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JOURNAL OF THE FISHERIES RESEARCH BOARD OF CANADA

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Fertilization and Predator Control to Increase Growth Rate and Yield of Trout in a Natural Lake[1]

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ABSTRACT

Enrichment in 1946 of Crecy Lake (20 ha; mean depth, 2.4 m), New Brunswick, with commercial fertilizers to provide 210 ppb of nitrogen (N), 390 ppb of phosphorus (P), and 270 ppb of potassium (K) had increased the growth rates of native and planted brook trout. In further studies, control of predation by fish-eating birds and mammals and reduction in number of eels, along with another application of fertilizers in 1951, improved the percentages of planted brook trout taken by anglers. Maximum percentages before and after predators were controlled were 17 and 88 for planted yearlings and 4 and 42 for planted underyearlings. Yields of planted trout to anglers and catches per unit effort improved commensurately. Autumn-stocked yearlings were largely (96%) angled in April before appreciable growth was made in the lake. Increases in yields of trout flesh produced in the lake resulted largely from the growth and higher survival of planted underyearlings. Harvested weights were greater than weights of plantings only when 16% or more of planted underyearlings were angled.

When underyearling and yearling rainbow trout were planted instead of brook trout, with a third fertilization in 1959 and continued predator control, they grew faster than brook trout of comparable size. Rainbow trout produced more fish flesh in the lake than brook trout when the percentage returns to anglers for the two species were similar. However, the percentage return to the anglers from most plantings of underyearling rainbow trout was low, with the net result that the rainbow trout did not provide as consistently good angling as the brook trout nor did they utilize the productive capacity of the lake more effectively.

Brook and rainbow trout spawned in the littoral areas of the lake. However, yields of naturally reared trout were poor, particularly when the survival of planted trout was high.

Killifish increased in numbers with fertilization, with or without predator control. Cyprinids were much less abundant than killifish, and sticklebacks disappeared from the lake.

INTRODUCTION

Inorganic fertilizers were added to Crecy Lake, Charlotte County, New Brunswick, in 1946 in an attempt to increase the growth and yield of brook trout, *Salvelinus fontinalis* (Mitchill), to anglers (Smith, 1955a). Growth of introduced hatchery-reared brook trout was improved. However, the consistent annual stocking appeared to attract additional predators (birds and mammals), and yields to anglers of planted trout declined. When stocking and effective predator control were applied simultaneously with a second fertilization in 1951, much improved yields to anglers of trout were realized.

This paper continues the reporting by Smith (1955a) on the growth and yields to anglers of brook trout with the second fertilization (1951) and continued stocking and predator control. Additionally, results of stocking rainbow

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trout, *Salmo gairdneri* Richardson, in place of the brook trout, with a third fertilization (1959) and continued predator control, are reported. An objective in stocking rainbow trout was to learn if this nonindigenous salmonid would utilize the productive capacity of the lake more effectively than the indigenous brook trout, and provide better yields to anglers.

STUDY AREA AND METHODS

Crecy Lake

Morphometry and hydrography of the lake have been described by Smith (1952, 1955a). Crecy Lake is a 50-acre (20 ha) headwater lake (67°01′W long, 45°14′N lat) in the Bocabec River System, which is tributary to Passamaquoddy Bay, southwestern New Brunswick. The waters of the lake are not thermally stratified in summer. Mean and maximum depths are 7.8 and 12.5 ft (2.4 and 3.8 m) respectively. The lake lies in an igneous rock formation (Silurian). Total hardness of water, as $CaCO_3$, is about 9 ppm. The water normally has a colour of about 5 ppm on the platinum-cobalt colour scale.

Summer water temperatures and late winter dissolved oxygen concentrations (under ice) in the lake are recorded in Tables I and II.

Table I. Summer water temperatures (C) of Crecy Lake, 1946–62. Numbers of readings given in parentheses.

Date	Surface		Bottom	
	Mean	Range	Mean	Range
June 1–15	17.5(19)	14.0–20.2	16.9(19)	13.9–19.5
16–30	18.9(23)	13.1–23.3	18.2(23)	12.1–23.3
July 1–15	21.3(25)	17.1–26.9	20.6(25)	17.0–23.0
16–31	22.0(26)	17.1–25.1	21.8(26)	16.9–24.4
Aug. 1–15	21.6(24)	18.9–25.7	21.1(24)	19.1–23.7
16–31	20.9(27)	18.9–22.6	20.5(27)	18.0–22.6
Sept. 1–15	19.3(16)	15.2–23.4	18.6(16)	16.3–22.9
16–30	16.2(14)	11.1–20.2	15.5(14)	11.0–18.6

Stocking with Hatchery-Reared Trout

The brook trout is indigenous to Crecy Lake. With no permanent tributary streams, spawning was limited to littoral areas of the lake and to restricted sections of the outlet. After the installation of a fish barrier (1948) in the outlet, native (naturally reared) brook trout in Crecy Lake came only from spawning in the lake. The anglers' catches of native trout in 1943 and 1944, before continued stocking began, numbered 166 and 148 respectively (Smith, 1955a). Stocking (see p. 2015) was undertaken to ensure a population of trout adequate to capitalize on possible increase in productive capacity of the lake with fertilization, and better survival with predator control.

TABLE II. Dissolved oxygen concentrations of Crecy Lake water at three depths under ice in March. Depths from surface of ice. No data for March in missing years.

Day in March and year	1 m		2 m		3 m (within 0.3 m of bottom)	
	ml/liter	*% sat.*	*ml/liter*	*% sat.*	*ml/liter*	*% sat.*
17, 1947	7.3	75	6.6	72	6.1	67
29, 1949	6.4	68	5.9	64	4.4	48
15, 1950	8.8	94	5.9	64	5.8	63
16, 1951	6.6	72	6.4	70	6.1	69
7, 1952	8.2	87	6.0	66	1.4	15
18, 1953	7.0	76	7.2	79	7.4	82
17, 1954	6.6	72	6.2	67	6.1	67
21, 1955	8.4	91	4.3	47	3.7	40
19, 1956	5.6	60	3.8	41	2.8	31
29, 1960	6.0	64	3.6	39	1.9	21
28, 1961	4.4	48	2.9	32	0.8	9
28, 1962	8.1	88	6.8	75	6.8	75

The trout were planted in the autumn. All were marked by removal of the adipose and one of the paired fins. The paired fins regenerated in varying degree, but their mutilation was recognized by the technician resident at the lake, who examined all angled trout for clipped fins. Numbers and sizes of hatchery-reared trout planted are given in Tables XI and XII. No trout were planted in 1957.

A trap to capture downstream-moving fish was incorporated into the outlet fish barrier in 1949. The barrier and trap were maintained throughout the year by the technician. Records of planted brook and rainbow trout that attempted to leave the lake and were captured in the trap are given by Smith (1967a).

FERTILIZATION

Commercial inorganic fertilizers were added to Crecy Lake on three occasions: June 19 and 20, 1946; June 20, 1951; July 16, 1959. In each case the fertilizers provided 210 ppb of nitrogen (N), 390 ppb of phosphorus (P), and 270 ppb of potassium (K) if equally distributed throughout the volume of the lake. The nature of the fertilizers in the first two applications has been described (Smith, 1955a).

The third application (July 16, 1959) of fertilizers consisted of the following materials: ammonium nitrate (33.5% N), 530 lb (1 lb = 0.45 kg); ammonium sulphate (21% N), 200 lb; triple superphosphate (46% P_2O_5), 1560 lb; normal superphosphate (20% P_2O_5), 1210 lb; potassium chloride (60% K_2O), 500 lb.

The larger proportion (75%) of the fertilizer was broadcast in dry form over the littoral areas of the lake up to about 1 m (3 ft) deep. The remainder

was spread over the deeper, central part of the lake. The third application was made later than in 1946 or 1951 in order to have approximately the same discharge of water from the lake at each application.

Predator Control

Fish-eating birds and mammals.— Annual stocking with trout appeared to attract increased numbers of fish-eating birds and mammals to the lake. It was suspected that favourable effects of the fertilization in 1946 (Smith, 1955a) were appreciably offset by increased depredations. Anglers' catches were reduced. Control measures against these predators began in 1949, and were continued to 1963. Action consisted of frightening as many as possible of the predators from the lake, and of destroying those that remained by gun and trap. The incidence and kill of fish-eating birds and mammals at the lake have been recorded (Smith, 1967b). Kingfisher, loon, osprey, great blue heron, merganser, mink, and otter were the most frequent visitors to the lake. None of the birds nested in the vicinity of the lake. Predator control was exercised by a resident technician.

Eels.— A second barrier was placed in the outlet of the lake in 1951 in an attempt to prevent the entrance of the young (elvers) of the catadromous American eel, *Anguilla rostrata* (LeSueur) and thereby reduce or eliminate predation and competition by this species with trout (Smith, 1955b). The barrier channelled all of the outflow over a sluice, to fall vertically about 2 ft into the stream bed below. The majority of eels at the lake spend 3 or 4 years in the lake, with lesser numbers up to 7 years (Smith and Saunders, 1955). Accordingly, reduction in numbers of eels in the lake from this action should occur gradually over a number of years.

Summer fishing with pots and set-lines was undertaken (1950–58) to reduce the number of eels already in the lake. However, most eels captured in the lake during the summer were those of a size and age that would have migrated from the lake in the autumn of the year of capture. Thus, this control measure was limited largely to the older eels and affected only a short period of their stay in the lake.

Older brook trout.— In 1954 predator control at Crecy Lake was extended by gillnetting 110 yearling (age I) and older trout in late summer and early autumn prior to the introduction of underyearlings (age 0) (Smith, 1956). This measure was undertaken only in 1954.

Creel Census

An annual creel census was made by the resident technician at Crecy Lake from 1943 through 1963, with the exception of 1945. Access to the lake is by a single road which terminates where the technician resided. Boats for fishing on the lake were under the control of the technician.

Angled trout were enumerated, and records kept of the fork length and weight of a majority of the trout. Records were also obtained of the number of rods and rod-hours spent in fishing, whether trout were taken or not, and

of finclipping pattern for each trout recaptured. All records were entered on creel census cards, one each for every angler or group of anglers in a boat.

The provincial angling season was from April 1 to September 15 through 1955. Thereafter the season was from April 15 to September 15. In 1956 only, the opening of the angling season was deferred in Crecy Lake to June 1 to learn if the summer quality of angling could be improved by conserving the stock against spring fishing (Smith, 1960).

No minimum size limit was applied. The bag limit permitted capture in 1 day of 10 lb of trout in aggregate, plus one fish, or 20 trout although this number might weigh less than 10 lb.

Chronology of Actions Taken at Crecy Lake

Stocking with hatchery-reared trout	
Brook trout — underyearlings	1940, 1946–56
— yearlings	1944–53
Rainbow trout — underyearlings	1958–62
— yearlings	1959–60
Stocking rate doubled	1951–56, 1958–62
Creel census	1943–44, 1946–63
Fertilization	1946, 1951, 1959
Fish barrier in outlet	1948–63
Fish trap in outlet barrier	1949–63
Predator control	
Fish-eating birds and mammals	1949–63
Eels	1951–61
Reduction in number of yearling trout	1954
Deferment of opening of angling season	1956

Methods of Angling

Most angling was from boats (Table III). Casting from the shore was difficult because of the thickly wooded shores and the shallowness of the littoral areas. Fishing from the ice into open water at the outlet, particularly in the late spring of 1961, was classed as from shore. Still-fishing from boats with earthworms or small fish (mainly killifish) as bait accounted for the greater part of the fishing effort. More trolling was done for rainbow than brook trout. The former appeared to be more wary of an anchored boat in shallow Crecy Lake than the latter.

The designation "rod" in Table III is for an angler fishing any part of a day. Numbers of anglers made several visits to the lake during an angling season.

GROWTH OF TROUT

Data on growth of brook trout in the plantings of 1951 and earlier are given by Smith (1955a). The growth rate improved with the first (1946) fertilization. An important result from the viewpoint of angling was that trout grew sufficiently well to be taken consistently by anglers when yearlings. This situation did not obtain in Crecy Lake before fertilization. When predator

TABLE III. Methods and numbers of rods used in angling for brook and rainbow trout, 1954–63.

Year	From shore		From boats			Season totals
	Bait	Fly	Still fishing with bait	Trolling with bait	Fly	
Brook trout						
1954	27	13	428	70	56	594
1955	48	14	545	16	29	652
1956	1	14	449	28	46	538
1957	1	13	734	35	29	812
1958	0	0	157	60	0	217
Total	77(3%)	54(2%)	2313(82%)	209(7%)	160(6%)	2813
Rainbow trout						
1959	0	0	112	84	29	225
1960	68	0	403	237	48	756
1961	106	1	641	198	99	1045
1962	2	1	482	174	69	728
1963	16	0	123	194	35	368
Total	192(6%)	2(<1%)	1761(56%)	887(28%)	280(9%)	3122
Grand total	269(5%)	56(1%)	4074(69%)	1096(18%)	440(7%)	5935

control was exercised along with fertilization (1951), yields to anglers were much improved. Concurrently, the growth rate of planted underyearling trout appeared to be depressed. However, the growth rate of planted underyearlings (1951–58) was correlated with size when planted ($r = 0.927$, $p < 0.01$).

To aid in assessment of the relative effects of these factors, as well as that of fertilization, on the growth rate of brook trout, the following regression equations were calculated from data (Tables IV–V; Smith, 1955a, tables III, IV) for the entire regime of fertilization (1946–57). For planted underyearlings,

$$Y = 0.2156 - 0.0142X_1 + 3.4063 \times 10^{-9}X_2 + 0.0139X_3 - 0.0344X_4$$

where Y is the monthly instantaneous growth rate by weight to capture by anglers in the year after planting (June), X_1 is fork length (centimeters) at planting, X_2 is biomass expressed by total weight of anglers' catches, X_3 is fertilization as measured by weight of bottom fauna (Smith, 1961, and unpublished data), and X_4 is predator control (0 for years without and 1 for years with control).

The coefficient of X_2 (biomass) is not significant ($p > 0.90$), but is highly correlated ($r = 0.788$) with that of X_4 (predator control). (Determination if coefficients could be zero was by the Student's t-test; probabilities, from a table of t values.) The coefficient of X_3 (fertilization) is also not significant ($p > 0.40$). When X_2 is eliminated the equation becomes

$$Y = 0.0896 - 0.0143X_1 + 0.0144X_3 - 0.0337X_4,$$

TABLE IV. Average fork lengths (cm) of brook trout when planted and when angled in 1st and 2nd years after planting, and monthly instantaneous growth rates (in parentheses) to month of capture, 1951–56, with predator control each year and fertilization in 1951. Stocking with brook trout discontinued in 1956. Dashes indicate no trout caught.

Planting date	Avg length when planted	Avg length when angled during 1st year						Avg length when angled during 2nd year					
		April	May	June	July	Aug.	Sept.	April	May	June	July	Aug.	Sept.
Planted as underyearlings (age 0)													
Sept. 7, 1951	7.9	14.5 (0.092)	15.6 (0.085)	16.1 (0.079)	17.0 (0.077)	18.7 (0.078)	–	24.7 (0.060)	23.9 (0.055)	26.9 (0.058)	–	30.0 (0.058)	–
" 5, 1952	6.7	14.3 (0.11)	16.4 (0.11)	17.0 (0.10)	17.6 (0.097)	17.8 (0.089)	17.9 (0.084)	20.7 (0.059)	22.0 (0.059)	22.7 (0.058)	24.2 (0.058)	27.1 (0.061)	–
" 9, 1953	7.5	14.5 (0.093)	15.3 (0.089)	15.7 (0.082)	15.7 (0.074)	16.3 (0.071)	17.3 (0.070)	22.0 (0.057)	23.7 (0.058)	24.3 (0.056)	–	–	–
" 8, 1954	10.8	15.2 (0.048)	15.8 (0.048)	17.4 (0.053)	18.0 (0.051)	20.0 (0.056)	–	–	–	27.3 (0.044)	28.2 (0.044)	28.8 (0.043)	–
" 8, 1955	8.5	–	–	15.1 (0.064)	14.9 (0.056)	15.9 (0.057)	17.9 (0.062)	23.4 (0.053)	22.3 (0.048)	27.8 (0.056)	25.4 (0.050)	–	–
" 13, 1956	9.5	14.3 (0.058)	14.3 (0.051)	14.9 (0.050)	16.8 (0.057)	17.8 (0.057)	19.5 (0.060)	26.6 (0.054)	27.3 (0.053)	29.4 (0.054)	–	–	–
Planted as yearlings (age I)													
" 5, 1951	17.3	20.9 (0.027)	20.8 (0.024)	21.7 (0.025)	–	25.6 (0.037)	–						
Nov. 2, 1951	24.8	27.3 (0.019)	27.1 (0.015)	26.3 (0.0084)	–	–	–						
Sept. 10, 1953	19.7	21.4 (0.012)	21.0 (0.0080)	21.1 (0.0076)	24.9 (0.023)	–	–						
Nov. 3, 1953	24.9	26.2 (0.010)	24.7	24.1	–	–	–						

TABLE V. Average weights (g) of brook trout when planted and when angled in 1st and 2nd years after planting, and monthly instantaneous growth rates (in parentheses) to month of capture, 1951–56, with predator control each year and fertilization in 1951. Stocking with brook trout discontinued in 1956. Dashes indicate no trout caught.

Planting date	Avg wt when planted	Avg wt when angled during 1st year						Avg wt when angled during 2nd year					
		April	May	June	July	Aug.	Sept.	April	May	June	July	Aug.	Sept.
Planted as underyearlings (age 0)													
Sept. 7, 1951	8.4	38.3 (0.22)	41.4 (0.20)	49.9 (0.20)	66.6 (0.21)	59.3 (0.18)	–	173.2 (0.16)	200.7 (0.16)	241.0 (0.16)	–	311.9 (0.16)	–
" 5, 1952	4.2	30.6 (0.28)	52.7 (0.32)	58.4 (0.29)	62.7 (0.27)	64.9 (0.26)	63.8 (0.23)	89.6 (0.16)	120.8 (0.17)	136.1 (0.17)	173.2 (0.17)	227.4 (0.17)	–
" 9, 1953	5.9	33.5 (0.25)	40.5 (0.24)	43.4 (0.22)	48.2 (0.21)	53.3 (0.20)	–	131.0 (0.16)	154.8 (0.16)	170.1 (0.16)	–	–	–
" 8, 1954	12.9	35.7 (0.15)	46.2 (0.16)	64.4 (0.18)	71.7 (0.17)	95.8 (0.20)	–	–	–	263.1 (0.14)	227.6 (0.10)	311.9 (0.14)	–
" 8, 1955	7.6	–	–	38.8 (0.18)	40.5 (0.17)	48.2 (0.17)	77.4 (0.18)	157.1 (0.16)	197.6 (0.16)	241.0 (0.16)	219.2 (0.15)	–	–
" 13, 1956	10.7	30.6 (0.15)	32.0 (0.13)	37.4 (0.14)	59.5 (0.17)	57.3 (0.15)	86.8 (0.17)	224.2 (0.16)	262.2 (0.16)	329.4 (0.16)	–	–	–
Planted as yearlings (age I)													
" 5, 1951	60.0	110.3 (0.087)	105.5 (0.071)	117.4 (0.075)	–	205.5 (0.11)	–						
Nov. 2, 1951	192.0	227.9 (0.034)	232.2 (0.032)	195.0 (0.0022)	–	205.5	–						
Sept. 10, 1953	97.6	94.1	95.5	99.2 (0.0018)	151.1 (0.043)	–	–						
Nov. 3, 1953	212.9	183.7	150.5	170.1	–	–	–						

which is similar to the original equation. The coefficient of X_3 (fertilization) remains not significant ($p > 0.30$), but the coefficients for length at planting and predator control are highly significant ($p < 0.001$). When X_3 is eliminated,

$$Y = 0.0896 - 0.0141X_1 - 0.0359X_4,$$

and the coefficients of X_1 and X_4 remain at the significantly high levels.

For planted yearlings,

$$Y = 0.03781 - 0.0154X_1 - 5.3476 \times 10^{-8}X_2 - 0.0123X_3 + 0.0051X_4.$$

All of the coefficients, except for length at planting (X_1), are not significant ($p > 0.80$). Again the coefficients of biomass (X_2) and predator control (X_4) are strongly correlated ($r = 0.872$). When X_4 is removed the significance of biomass remains at a low level ($p > 0.50$). Significance of fertilization (X_3) is low ($p > 0.90$) and that for length at planting is high ($p < 0.01$).

Among the factors considered, the relatively high significance of the coefficients for size at planting and for predator control (increased number of fish) shows a dominating influence of these factors on the growth rate of planted underyearlings. The growth rate was higher ($p > 0.01$) before predator control, in line with the negative term for predator control (X_4). There remained a positive effect from fertilization, however, even though predator control depressed the growth rate.

The slower growth rate of planted underyearlings with predator control may in large measure be attributed to intraspecific competition for a common food supply by a larger number of fish. Such a relationship was not evident for the trout planted as yearlings. The majority of these, however, were angled in April, presumably before effects of competition for food may have become an important factor affecting their growth rate.

Rainbow trout when planted as underyearlings grew sufficiently well with the third (1959) fertilization to be angled when yearlings (Tables VII and VIII). When planted as yearlings in autumn they made appreciable growth by the time they were angled during the following April and May. As noted, on the average brook trout planted as yearlings lost weight over winter (Table V).

Instantaneous rates of growth by weight of the planted rainbow trout (Table VIII), as with brook trout, were strongly correlated with size at planting ($r = 0.881$, $p < 0.01$). Number and biomass, measured by yields to anglers, had some effect on the growth rate of rainbow trout, but for the limited data available the correlations between number and biomass on the one hand and instantaneous growth rate on the other were weak (with number, $r = -0.430$; with biomass, $r = -0.370$; $p > 0.10$ for both). Burdick and Cooper (1956) reported a slower growth rate of planted underyearling rainbow trout in Weber Lake, Wisconsin, when the percentage taken by anglers was high (18.3%) than when the percentage was low (0.3–5.9%).

In general, rainbow trout grew faster than brook trout in Crecy Lake. To illustrate, the average instantaneous growth rates by weight of the two

species, planted as underyearlings and angled as yearlings, were compared by pairing the data for years in which the sizes at planting were least divergent: 1958 with 1952, 1960 with 1954, and 1961 with 1956 (Tables V and VIII). Differences in means of the monthly instantaneous growth rates (weighted with respect to the number of months that the fish were in the lake before capture) are highly significant at $p > 0.01$. Poorer survival, and thus less dense populations, may have favoured a faster growth rate by the rainbow trout in certain years, but regression analyses failed to demonstrate that density of the rainbow trout populations had a significant effect on growth rate. More use by rainbow trout than brook trout of micro-crustacean plankters as food (Smith, unpublished data) may have been a factor in a faster growth rate by the former.

YIELDS TO ANGLERS

Numbers and Weights

The average annual yield of brook trout by number from 1952 through 1957 was 3833 fish (range 2418–5623) (Table VI). The 1958 catch was 161 fish, no trout having been planted in 1957. The peak year was 1955 when 5623 trout were caught. Removal of yearling trout in 1954 (Smith, 1956) and a lower number of eels in the lake (Smith, 1966), in addition to control of fish-eating birds and mammals, probably contributed to the peak yield. The lower yield in 1956 (2989 trout) coincided with deferment of the opening of the angling season from April 15 to June 1 (Smith, 1960). The objective of this action was to provide better angling in the later part of the season by protection of trout against the spring fishing. The June catch was especially better than in other years between 1952 and 1957, but not those in July and August. The action proved abortive insofar as the annual yield for the shortened season and the number caught per rod-day fell.

The average annual yield of brook trout by weight from 1952 through 1957 was 615 lb (range 309–914 lb) or 12.2 lb per acre (13.7 kg per ha) (Table VI). The year (1953) for peak weight did not coincide with that (1955) for number. The highest yields by weight were obtained when the percentages of planted yearlings taken by anglers (Table XI) were highest.

Data for the number and weight of brook trout taken by anglers before and after the first fertilization (1946) and before predator control was applied (1949) were reported by Smith (1955a). In 1947 number and weight of trout in the catches increased 2.8 and 2.1 times respectively. The modest higher yield in absolute quantity was not sustained, however, and catches were below pre-fertilization levels in 1948 and 1949. As already noted, observations indicated an increased number of fish-eating birds and mammals foraging at the lake in these latter years. The yields by number and weight increased sharply in 1951 after predator control became effective (Smith, 1955a). The catch in 1951 was largely from the first stocks of planted trout receiving year-round protection from predators. The increase in 1951 was from the same level of stocking as in previous years (Smith, 1955a) and, for the most part,

TABLE VI. Yields to anglers of brook and rainbow trout by number and weight from Crecy Lake, 1952–63 (1 lb = 0.4536 kg; lb per acre × 1.12 = kg per ha; lb per 10^6 ft^3 × 16.02 = kg per 10^6 m^3).

Year	No. caught	No. of rod-days	No. per rod-day	No. of rod-hours	No. per rod-hour	Wt		
						lb	*lb/acre*	*lb per 10^6 ft^3*
				Brook trout				
1952	2418	447	5.4	1490	1.6	572.9	11.4	33.3
1953	3276	553	5.9	1688	1.9	914.3	18.1	53.2
1954	4073	594	6.9	1576	2.6	648.8	12.9	37.8
1955	5623	652	8.6	1737	3.2	532.4	10.6	31.0
1956	2989	538	5.6	1569	1.9	309.3	6.1	18.0
1957	4616	812	5.7	2343	2.0	411.3	8.2	23.9
1958	161	217	0.7	527	0.3	86.1	1.7	5.0
				Rainbow trout[a]				
1959	218	225	1.0	519	0.4	52.2	1.0	3.0
1960	1482	756	2.0	2115	0.7	512.7	10.2	29.8
1961	4199	1045	4.0	3587	1.2	772.2	15.4	44.9
1962	853	728	1.2	2153	0.4	279.6	5.5	16.2
1963	79	368	0.2	923	0.1	59.2	1.2	3.4

[a]Some brook trout included; see Tables XI and XIII.

TABLE VII. Average fork lengths (cm) of rainbow trout when planted and when angled in 1st, 2nd, and 3rd years after planting, and monthly instantaneous growth rates (in parentheses) to month of capture, 1958–62, with predator control each year and fertilization in 1959. Dashes indicate no trout caught.

Planting date	Avg length when planted	Month of angling					
		April	May	June	July	Aug.	Sept.
Planted as underyearlings (age 0) Angled during 1st year							
Sept. 8, 1958	6.4	–	15.1 (0.11)	17.9 (0.11)	18.1 (0.10)	22.3 (0.11)	23.4 (0.11)
Oct. 6, 1959	7.6	–	19.0 (0.13)	16.6 (0.095)	15.5 (0.079)	20.3 (0.098)	21.2 (0.084)
" 4, 1960	10.6	15.5 (0.063)	16.5 (0.063)	17.9 (0.065)	18.2 (0.060)	19.3 (0.060)	19.9 (0.057)
" 11, 1961	9.2	13.7 (0.066)	13.3 (0.052)	15.3 (0.069)	18.9 (0.080)	19.7 (0.076)	20.7 (0.074)
" 2, 1962	8.9	–	14.5 (0.070)	15.9 (0.073)	17.6 (0.076)	18.4 (0.073)	20.0 (0.074)
Angled during 2nd year							
Sept. 8, 1958	6.4	24.0 (0.070)	24.8 (0.068)	25.1 (0.065)	27.9 (0.067)	28.4 (0.065)	28.5 (0.062)
Oct. 6, 1959	7.6	24.6 (0.065)	24.7 (0.062)	26.8 (0.063)	32.2 (0.069)	30.4 (0.063)	–
" 4, 1960	10.6	22.4 (0.042)	23.1 (0.041)	23.9 (0.041)	27.0 (0.045)	27.7 (0.044)	28.4 (0.043)
" 11, 1961	9.2	–	27.3 (0.057)	26.9 (0.054)	27.2 (0.052)	29.7 (0.049)	–
Angled during 3rd year							
Sept. 8, 1958	6.4	32.1 (0.052)	31.7 (0.050)	32.3 (0.049)	–	–	–
Oct. 6, 1959	7.6	31.7 (0.050)	33.9 (0.054)	33.9 (0.052)	35.1 (0.052)	31.4 (0.046)	–
" 4, 1960	10.6	–	33.2 (0.037)	31.4 (0.034)	35.0 (0.036)	34.2 (0.035)	33.6 (0.033)
Planted as yearlings (age 1) Angled during 1st year							
" 16, 1959	19.7	23.3 (0.028)	23.7 (0.026)	24.1 (0.025)	26.4 (0.032)	27.4 (0.033)	27.9 (0.032)
" 4, 1960	17.6	21.1 (0.030)	20.8 (0.024)	22.5 (0.031)	22.2 (0.026)	23.1 (0.027)	22.9 (0.024)
Angled during 2nd year							
" 16, 1959	19.7	30.7 (0.025)	31.3 (0.024)	31.4 (0.023)	32.2 (0.023)	31.5 (0.021)	30.4 (0.019)
" 4, 1960	17.6	25.6 (0.021)	25.1 (0.019)	25.1 (0.018)	29.7 (0.025)	27.7 (0.021)	28.5 (0.021)

TABLE VIII. Average weights (g) of rainbow trout when planted and when angled in 1st, 2nd, and 3rd years after planting, and monthly instantaneous growth rates (in parentheses) to month of capture, 1958–62, with predator control each year and fertilization in 1959. Dashes indicate no trout caught.

Planting date	Avg wt when planted	Month of angling					
		April	May	June	July	Aug.	Sept.
Planted as underyearlings (age 0) Angled during 1st year							
Sept. 8, 1958	3.3	–	43.7	67.5	79.9	152.5	149.7
			(0.32)	(0.34)	(0.32)	(0.35)	(0.32)
Oct. 6, 1959	6.0	–	70.3	56.1	35.4	99.2	163.0
			(0.35)	(0.28)	(0.20)	(0.28)	(0.30)
" 4, 1960	17.7	36.9	50.2	62.4	65.2	79.4	87.9
		(0.12)	(0.15)	(0.16)	(0.14)	(0.15)	(0.15)
" 11, 1961	12.2	21.3	24.5	42.5	77.1	97.0	104.0
		(0.093)	(0.10)	(0.16)	(0.20)	(0.21)	(0.19)
" 2, 1962	9.5	–	35.4	46.2	58.1	63.8	71.0
			(0.19)	(0.20)	(0.20)	(0.19)	(0.18)
Angled during 2nd year							
Sept. 8, 1958	3.3	155.4	173.5	193.9	253.4	278.7	271.3
		(0.20)	(0.20)	(0.19)	(0.20)	(0.19)	(0.18)
Oct. 6, 1959	6.0	155.9	180.9	208.7	355.8	212.6	–
		(0.18)	(0.18)	(0.18)	(0.19)	(0.17)	
" 4, 1960	17.7	115.4	131.5	152.8	226.2	250.0	258.3
		(0.10)	(0.11)	(0.11)	(0.12)	(0.12)	(0.12)
" 11, 1961	12.2	297.7	231.6	236.2	252.9	325.9	–
		(0.18)	(0.16)	(0.15)	(0.14)	(0.15)	
Angled during 3rd year							
Sept. 8, 1958	3.3	389.8	358.1	389.0	–	–	–
		(0.15)	(0.15)	(0.14)			
Oct. 6, 1959	6.0	332.3	415.9	441.7	432.3	311.9	–
		(0.13)	(0.14)	(0.13)	(0.13)	(0.12)	
" 4, 1960	17.7	–	474.9	357.8	505.0	484.2	463.0
			(0.10)	(0.091)	(0.10)	(0.097)	(0.093)
Planted as yearlings (age I) Angled during 1st year							
" 16, 1959	95.9	133.8	149.1	150.0	205.0	229.9	247.5
		(0.056)	(0.063)	(0.056)	(0.085)	(0.087)	(0.086)
" 4, 1960	69.6	97.5	97.0	106.9	123.9	129.8	132.1
		(0.056)	(0.048)	(0.053)	(0.064)	(0.062)	(0.058)
Angled during 2nd year							
" 16, 1959	95.9	324.9	353.0	343.6	358.6	337.9	326.0
		(0.068)	(0.069)	(0.064)	(0.057)	(0.063)	(0.053)
" 4, 1960	69.6	162.4	162.7	174.6	301.4	223.4	248.1
		(0.049)	(0.045)	(0.046)	(0.070)	(0.051)	(0.055)

showed the favourable result in yield of the control of this type of predation alone. Presumably, doubling the stocking rate in the autumn of 1951 contributed to the higher catches in 1952 and later, but predator control remained a dominant factor in the much greater yields.

Annual yields of rainbow trout to anglers ranged widely (Table VI) when this species supplanted brook trout in the stocking (1958–62), with a third fertilization of the lake and continued predator control. The yield in 1961 compared favourably with best yields of brook trout (1954 and 1955). In other years, however, the yields of rainbow trout were lower, particularly in 1959 and 1963, when they were of the order realized (Smith, 1955a) from plantings of brook trout with the first fertilization without predator control.

Trout Flesh Produced in the Lake

With the second fertilization (1951), effective predator control (1951–57), and continued planting of brook trout (1951–56) the annual yields from 1952 to 1957 of trout flesh produced in the lake averaged 62% (range 48–83%) of the total yields (Table IX). Yields of trout flesh produced in the lake in

Table IX. Yields to anglers of trout flesh produced in Crecy Lake, 1952–63 (1 lb = 0.4536 kg; lb per acre × 1.12 = kg per ha).

Year	Wt of native trout (brook trout) (*lb*)	Increase in wt of angled planted trout (*lb*)	Total wt produced in lake		% of total wt of trout angled from lake
			lb	*lb/acre*	
Brook trout planted (except in 1957)					
1952	3.4	273.5	276.9	5.5	48
1953	5.0	432.9	437.9	8.7	48
1954	2.3	318.6	320.9	6.4	49
1955	3.2	347.6	350.8	7.0	66
1956	3.0	253.5	256.5	5.1	83
1957	1.0	308.7	309.7	6.1	75
1958	2.8	79.7	82.5	1.6	96
Rainbow trout planted					
1959	18.3	32.7	51.0	1.0	98
1960	16.2	235.7	251.9	5.0	49
1961	11.3	579.6	590.9	11.8	77
1962	4.5	235.7	240.2	4.8	86
1963	6.5	45.5	52.0	1.0	88

those years came almost entirely from growth of planted underyearlings. Planted yearlings made little or no growth in the lake before they were caught (Tables IV and V). Angling for these fish proved essentially to be on a put-and-take basis, with the lake serving mainly as a holding area. The yields of brook trout flesh produced in the lake after the first fertilization, without

predator control, were comparatively low but they made up 82% of the total yields (Smith, 1955a). This was a period when the percentages of planted trout taken by anglers were low (0.4–19.8), and native trout made up substantial proportions of the catches.

The yield of rainbow trout in 1961 contained the most trout flesh produced in the lake in any year, whether the stock was brook or rainbow trout (Table IX). Also in 1960 and 1962 the increases in weight of rainbow per acre compared favourably with those of brook trout, but in 1959 and 1963 they were comparatively poor. Although there was no day-to-day creel census in 1964, records are adequate to show that catch of rainbow trout in that year, and later, was small. The yield from the planting of underyearling rainbow trout in 1962 would not be substantially increased by later captures.

The yield of rainbow trout flesh produced in the lake (Table IX) was favoured by generally higher percentages than for brook trout of planted fish taken by anglers in the 2nd and 3rd years after planting (Tables XI, XII). Thus, for example, underyearling rainbow trout planted in 1958 contributed 10% of the 1961 yield of trout flesh produced in the lake, although they numbered only 44 in a total catch of 4199 trout. Few planted brook trout were taken by anglers during the 3rd year after planting (Table XI). The largest trout of either species taken by anglers were rainbow trout which were planted as underyearlings and survived into the 3rd year after stocking. Potentially, therefore, if survival were equable, rainbow trout with a faster growth rate and a longer life span could surpass comparable plantings of brook trout in the amount of flesh produced in stocked waters.

Comparison of Planted and Harvested Weights

The total weight of underyearling and yearling brook trout planted (1945–56) was 4047 lb (Table X). Anglers harvested 4018 lb from the plantings. With yearlings there was a loss from 2149 lb in planted to 1568 lb in harvested weight. However, with underyearlings there was a gain from 1899 to 2450 lb. Gains in harvested over planted weights occurred almost entirely in the years when predator control was effective. Yields of planted underyearlings to anglers (Table XI) were 16% or higher when the harvested exceeded the planted weight.

When the opening of the angling season was deferred in 1956 from April 15 to June 1, the gain in weight (106 lb, Table X) from the underyearling planting in 1955 was less than the gains (118–187 lb) from comparable plantings in the 4 previous years without deferment of the angling season. Eipper (1964) found for unfished farm ponds in New York that the maximum biomass from autumn plantings of brook trout underyearlings was attained during the autumn a year later, when the trout were age I+. In general the brook trout is a short-lived species. It seems unlikely that better yields by weight from plantings of underyearlings at Crecy Lake would have been realized by later opening of the angling seasons, as done in 1956, or deferment of exploiting planted stocks until age II.

TABLE X. Weights of trout (lb) in plantings from 1945 to 1962 and yields (lb) to anglers from the plantings (1 lb = 0.4536 kg). Data for 1945–51 included as they were not previously reported in this form.

Year	Underyearlings planted			Yearlings planted		
	Planted wt	Angled wt	Gain or loss	Planted wt	Angled wt	Gain or loss
			Brook trout			
1945	–	–	–	148.6	42.5	–106.1
1946	103.4	104.9	1.5	170.0	68.0	–102.0
1947	82.2	30.6	–51.6	175.6	22.0	–153.6
1948	47.8	9.4	–38.4	71.4	10.1	–61.3
1949	61.4	46.7	–14.7	115.9	118.1	2.2
1950	132.4	226.4	94.0	136.9	125.9	–11.0
1951	262.4	130.0	–132.4	374.6	338.6	–36.0
1952	122.5	309.1	186.6	493.9	557.7	63.8
1953	174.2	299.7	125.5	462.0	285.0	–177.0
1954	377.0	534.2	157.2	–	–	–
1955	229.3	347.1	117.8	–	–	–
1956	306.1	411.9	105.8	–	–	–
			Rainbow trout			
1958	96.2	150.5	54.3	–	–	–
1959	184.2	60.7	–123.5	329.9	566.1	236.2
1960	522.4	557.4	35.0	201.1	239.5	38.4
1961	361.1	24.8	–336.3	–	–	–
1962	270.4	2.5	–267.9	–	–	–
Total	1434.3	795.9	–638.4	531.0	805.6	274.6

A gain in harvested over planted weight was realized from plantings of yearling but not underyearling rainbow trout (Table X). This was largely the reverse of the situation with brook trout during the period 1952–57, when the lake for the most part was treated similarly with respect to stocking, fertilization, and predator control. The best gain among rainbow trout plantings was that of yearlings planted in 1959. The gain in harvested over planted weight amounted to 4.7 lb per acre (5.2 kg per ha). There was a return to the angler in this instance of 1.7 lb for each pound planted. A more favourable result (4.4 lb) from six plantings of underyearling rainbow trout in a Wisconsin lake was reported by Burdick and Cooper (1956).

PERCENTAGES OF PLANTED TROUT TAKEN

With both fertilization (1951) and effective predator control (1951–59), the percentages of brook trout planted as underyearlings that were taken by anglers ranged from 11.7 to 41.9%, and for yearlings, from 36.2 to 88.4% (Table XI). With fertilization (1946), but without predator control (1946–48), the percentage returns from plantings of underyearling brook trout varied

TABLE XI. Numbers of brook trout, planted at two ages,[a] that were captured by anglers in various years at Crecy Lake after predators were controlled, 1952–59. Ages and average fork lengths (cm) when planted are given below the years of planting.

	Year of planting											
	1951			1952			1953			1954	1955	1956
Year of capture	*u* 7.9	*y* 17.3	*y* 24.8	*u* 6.7	*y* 20.8	*y* 26.0	*u* 7.5	*y* 19.7	*y* 24.8	*u* 10.8	*u* 8.5	*u* 9.5
1952	1,317	240	550									
1953	333	4	2	1,746	597	574	–	–	–	–	–	–
1954	6	–	–	417	–	5	2,707	377	536	–	–	–
1955	–	–	–	–	–	–	128	–	–	5,471	–	–
1956	–	–	–	–	–	–	4	–	–	69	2,990	–
1957	–	–	–	–	–	–	–	–	–	1	231	4,349
1958	–	–	–	–	–	–	–	–	–	–	–	155
1959	–	–	–	–	–	–	–	–	–	1	–	3
Total	1,656	244	552	2,163	597	579	2,839	377	536	5,542	3,221	4,507
No. planted	14,160	675	674	13,438	675	675	13,390	675	675	13,230	13,650	12,970
% recaptured by anglers	11.7	36.2	81.9	16.1	88.4	85.8	21.2	55.9	79.4	41.9	23.6	34.7

[a] *u* — planted as underyearlings (age 0); *y* — planted as yearlings (age I).

TABLE XII. Numbers of rainbow trout, planted at two ages,[a] that were captured by anglers at Crecy Lake with predators controlled, 1959–63. Ages and average fork lengths (cm) when planted are given below the years of planting.

	Year of planting						
		1959		1960			
	1958					1961	1962
	u	*u*	*y*	*u*	*y*	*u*	*u*
Year of capture	6.4	7.6	19.7	10.6	17.6	9.2	8.9
1959	156						
1960	177	33	1,238	–	–	–	–
1961	44	48	168	2,855	942	–	–
1962	1	25	19	583	56	165	–
1963	2	2	1	24	2	14	22
Total	380	108	1,426	3,462	1,000	179	22
No. planted	13,350	13,300	1,561	13,420	1,350	13,460	13,360
% recaptured by anglers	2.8	0.8	91.4	25.8	74.1	1.3	0.2

[a]*u* — planted as underyearlings (age 0); *y* — planted as yearlings (age I).

from 0.4 to 4.5%, and of yearlings, from 1.9 to 16.8% (Smith, 1955a). (Predator control was only partially effective in 1949, when percentage returns were 1.9 for underyearlings and 28.5 for yearlings (Smith, 1955a).)

The favourable effect of predator control on returns of planted brook trout was evident. Benefits of fertilization, acting primarily through an improved growth rate, were not so apparent. Predator control, presumably by improving survival and increasing the number of trout in the lake, reduced the growth rate as we have already seen. However, the growth remained sufficiently good with predator control that yearlings, planted as underyearlings, were of a size acceptable to anglers, and were well cropped before natural mortalities took a greater toll of their numbers. Without fertilization and predator control, percentages of planted underyearling brook trout taken by anglers in neighbouring lakes, as well as in other lakes in the Maritime Provinces, where assessment of stocking was made by creel censuses, were poor (0.1–2.6) (Smith, 1948a, 1952).

Percentages of planted yearling rainbow trout taken by anglers (Table XII) were comparable with those of yearling brook trout (Table XI). When underyearlings were planted, however, the returns of rainbow trout from four of the five plantings were much lower (0.2–2.8%). From the remaining planting, in 1960, the percentage return (26) compared favourably with the average return (25) realized from the six plantings of underyearling brook trout from 1951 to 1956.

Size at planting was a factor in the percentage taken by anglers, as witnessed by the much greater returns of yearlings for both rainbow and brook trout. With plantings of underyearling rainbow trout alone, however, size

at planting and percentage returns were poorly associated ($r = 0.630$; $p > 0.10$), even though the best return was obtained from the largest underyearlings planted (10.6 cm in 1960) (Table XII).

Wales and Borgeson (1961) also found wide variation in the percentage caught by anglers (2.1–29.3) from 11 plantings of marked underyearling rainbow trout in Castle Lake, California. In general, they found that the percentage of underyearlings caught was inversely correlated with population density of older rainbow trout in the lake and suggested cannibalism as a factor in the survival of underyearlings. Cannibalism may well have been a factor in the survival of planted underyearling rainbow trout in Crecy Lake as well, but the percentage of fish planted as underyearlings that were taken by anglers was highest in 1961, when the population of older trout was apparently second highest (Table XII). Also, the percentage return to anglers from the initial planting of underyearling rainbow trout (1958) was poor although the fish were subject to predation from only a relatively small population of older brook trout in the lake at that time.

Increased eutrophication of Crecy Lake, induced by artificial fertilization, and particularly manifest by algal blooms (Smith, 1948b and unpublished data), resulted in depressions of dissolved oxygen in the water under ice in late winter and early spring (Table II). The lethal concentration of dissolved oxygen for rainbow and brook trout is about 1.5 ppm (Burdick et al., 1954; Shephard, 1955). Inactivity and acclimation lower this concentration somewhat at winter water temperatures. However, the well-being of the trout is impaired at much higher levels. Graham (1949) found that concentrations of dissolved oxygen below 75% air saturation reduced activity of underyearling brook trout at all temperatures. It is assumed that rainbow trout would be affected in much the same way. Thus, the relatively low oxygen values of late winter in certain years could have adversely affected survival of planted trout. A causal relationship is again obscure since the apparently high survival from plantings of underyearling and yearling rainbow trout in 1960 (Table XII) followed the lowest recorded oxygen levels (Table II). The unanswerable question is whether survival would have been better with higher oxygen content. Our data provide no consistent explanation either of the apparently low survival of underyearling rainbow trout in most years, or for the difference between rainbow and brook trout in this regard under much the same conditions in the lake.

Contributions by Native Trout

Herein native trout are those from natural spawning, either by indigenous or introduced stock.

Native brook trout made minor contributions to the anglers' catches after stocking, fertilization, and predator control were applied together (1951–57) (Table XIII). The average annual catch of brook trout from 1951 through 1963 was 20 (range 4–58). Numbers of trout taken in 1943 and 1944, 166 and 148 (Smith, 1955a), indicated the angling possibilities with only natural reproduction, without fertilization and predator control.

TABLE XIII. Numbers and weights (lb) of native (naturally reared) brook trout angled, and percentages of annual anglers' catches of all trout from Crecy Lake, 1952–63 (lb = 0.4536 kg).

	No. caught		Wt	
Year	Native trout	% of all trout	Native trout	% of all trout
1952	14	0.6	3.4	0.6
1953	15	0.5	5.0	0.5
1954	26	0.6	2.3	0.4
1955	25	0.4	3.2	0.6
1956	6	0.2	3.0	1.0
1957	8	0.2	1.0	0.2
1958	4	2.5	2.8	3.3
1959	58	26.6	18.3	35.1
1960	25	1.7	16.2	3.2
1961	13	0.3	11.3	1.5
1962	7	0.8	4.5	1.6
1963	12	15.2	6.5	11.0

From 1948 through 1963, when spawning by trout was restricted to the lake, brook trout spawned successfully in the littoral areas of the lake at sites where a mixture of sand and gravel permitted redd formation. Usually there was seepage from contiguous low-lying shore areas. Annual estimates (1948–53) of native brook trout underyearlings, based on counts along the shore where they congregated during the first month after emergence from the redds, ranged from <1000 to 5000 (Smith, 1955a). Actions taken at Crecy improved angling for planted trout, but depressed that for native brook trout. It is suspected that predation by larger planted trout was an important factor in the poor survival of native trout. Evidence of this was the finding of native underyearlings in stomachs of angled, planted trout, usually in June. The comparatively high catch of native brook trout (58) in 1959 may have resulted from release of population pressure from introduced stock between the last planting of brook trout in 1956 and the first of rainbow trout in the autumn of 1959.

Rainbow trout also spawned in littoral areas of the lake in spring. Successful spawning of rainbow trout in lakes is apparently exceptional (McAfee, 1966). Developing eggs were found in redds on May 24, 1961. However, unshed eggs were found in a number of angled rainbow trout during the summer and early autumn (1961–63). Natural spawning contributed little to the anglers' catches. Only two naturally spawned rainbow trout were angled (1962). They were yearlings.

ANGLING

SEASON OF CAPTURE

A majority of the brook trout in the annual catches was taken in spring and early summer — 48% in April, 29% in May, and 13% in June (Fig. 1).

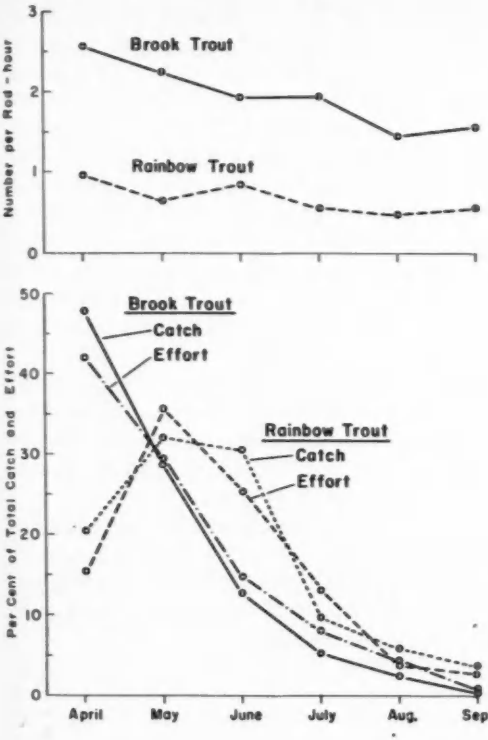

FIG. 1. Catch and effort (rod-hours) as percentages of annual totals, and catches per rod-hour, by months for brook and rainbow trout.

Of the trout planted as yearlings, 96% of the angling recaptures were made in April, with almost 100% by the end of June. In the case of underyearlings, 35% of the recaptures were made in April and 86% by the end of June.

A factor in the relatively poor catches of brook trout after June was the reduced supply in the lake after the heavy spring and early summer exploitation by anglers. The supply of planted yearlings was apparently almost exhausted by each spring angling. Few were taken in the summer of the year after planting. None was captured by netting in September (Smith, 1956), and of 4049 planted from 1951 to 1953 only 11 were angled during the second spring from planting. However, appreciable numbers of planted underyearlings escaped the early-season angling of their 1st year in the lake. Catches in July, August, and September (10% of the total) consisted mostly of these fish. Also, survival through the first summer in the lake was shown from netting in early autumn after close of the angling season (Smith, 1956), from population estimates by a marking and recapture procedure (Smith, 1960), and by being angled at age II+ in the spring of their 2nd year in the lake (Table XI). Estimated numbers of trout at the close of the angling season (preponderantly from underyearling plantings of the previous autumn) were 1400 in 1955 and 2000

in 1956 (Smith, 1960). The catchability of trout was poor in the warm water of summer and, from experience, anglers applied less angling pressure. Hoar (1942) and Baldwin (1957) observed that the feeding activities of brook trout were less, or ceased, as water temperatures approached and exceeded 20 C. Water temperatures of Crecy Lake in summer (Table I) commonly exceeded 20 C but were at times below this level. Although not systematically recorded, angling success was comparatively good during such periods, and largely accounted for the summer catch of brook trout. In summary, decline in catches with advance of the angling season was not only conditioned by a reduced supply of brook trout, but also by the adverse effect of summer water temperature on the catchability of those trout that survived into the summer period. If catches had been consistently good in summer, doubtless anglers would have applied more effort at that season.

Most rainbow trout were angled in May and June whereas the greatest proportion of brook trout catches came in April (Fig. 1). Amount of fishing effort was a factor in the difference. Anglers appeared reluctant to fish rainbow trout in the often inclement weather of April at the lower rate of capture than that previously experienced with brook trout (Fig. 1).

The percentages of the season's effort and catch of rainbow trout, in common with those for brook trout, were relatively low in July and August. Probably the important conditioning factors were the same as for brook trout, namely a decline in population from angling with advance of the angling season, and poorer catchability at summer water temperatures.

Angling Success

A high proportion of the anglers were successful in taking brook trout when Crecy Lake was stocked and fertilized and predator control was effective (Table XIV). A total of 3939 rod-days were recorded from 1952 through 1957. The number of successful rod-days, that is days on which one or more trout were captured, was 3465 (88%). The total number of rod-hours of effort in the same period was 11,412. The number of these involved in successful rod-days was 10,496 (92%).

Catches per rod-day and per rod-hour of brook trout (Table VI) were higher ($p < 0.01$) from 1952 through 1957, after predator control became effective, than in previous years (Smith, 1955a). The higher rates reflect the greater number of brook trout available to anglers. It is apparent that the actions taken at the lake much improved the angling success.

The percentage of successful rod-days in fishing rainbow trout was definitely lower in most years than for brook trout (Table XIV). A similar situation obtained with respect to catch per rod-hour (Table VI, Fig. 1). In 1961, when the rainbow trout population was apparently highest, 80% of the rod-days were successful and catches were made at a rate of 1.2 per rod-hour. This was the highest rate of capture realized for rainbow trout. In comparison, for the years 1952 through 1957, the percentage of successful

TABLE XIV. Percentages of rod-days and rod-hours that were successful (those with capture of one or more trout), 1952–63.

Year	Rod-days		Rod-hours	
	Total	Successful (%)	Total	% involved in successful rod-days
1952	447	84	1,490	92
1953	553	90	1,688	93
1954	594	88	1,576	91
1955	652	92	1,737	93
1956	538	92	1,569	92
1957	812	84	2,343	91
1958	217	40	527	55
1959	225	40	519	48
1960	756	65	2,115	73
1961	1,045	80	3,587	84
1962	728	50	2,153	61
1963	368	17	923	19

rod-days in fishing brook trout (Table XIV) ranged from 84 to 92 (average 88), and catch per rod-hour (Table VI) from 1.6 to 3.2 (average 2.1).

The disparity in rates of capture between the two species may in part be ascribed to differences in survival of planted stocks. However, in the 2 years 1954 and 1961, when comparable numbers of brook and rainbow trout were caught and when it may be reasonably assumed that populations in the lake were of the same order, the rate of capture for the former species was 2.6 and for the latter 1.2 fish per rod-hour (Table VI). It was the anglers' experience that rainbow trout were wary of anchored boats, from which much still-fishing with bait was done. As a result, a larger proportion of the effort was expended in trolling and fly-fishing for rainbow trout than previously for brook trout (Table III). Rainbow trout were captured in 1961 at a rate of 1.0 fish per rod-hour by still-fishing from anchored boats and at a somewhat higher rate of 1.4 per rod-hour by trolling and fly-fishing. Rainbow trout were more difficult to catch than brook trout by any of the modes of angling employed at Crecy Lake.

OTHER SPECIES OF FISH

The following species occurred at Crecy Lake in addition to the trout: redbelly dace (*Chrosomus eos* Cope), lake chub (*Hybopsis plumbea* (Agassiz)), blacknose dace (*Rhinichthys atratulus* (Hermann)), creek chub (*Semotilus atromaculatus* (Mitchill)), white sucker (*Catostomus commersoni* (Lacépède)), american eel (*Anguilla rostrata* (LeSueur)), banded killifish (*Fundulus diaphanus* (LeSueur)), threespine stickleback (*Gasterosteus aculeatus* Linnaeus), ninespine stickleback (*Pungitius pungitius* (Linnaeus)).

Redbelly dace were most numerous in 1953 and 1954. They were scarce before and after these years; in some years none was taken in traps or by seining. Lake chub, creek chub, and suckers were taken only once or twice, in the early years of the study. Blacknose dace were common in the area adjacent to the outlet. Sticklebacks decreased in numbers until none was observed in the lake or found in trout stomachs after 1952. Upstream recruitment to the lake by the above species was prevented by a trap maintained in the outlet from 1948 onward.

As a predator-control measure, an attempt was made to prevent the entrance of young eels (elvers) into the lake by a barrier in the outlet (Smith, 1955b). Results were gauged by the number of maturing eels trapped each year in the outlet as they migrated from the lake, and by capture in pots and setlines in summer in the lake (Smith, 1966). Those taken in the lake in summer were almost entirely of sizes that would have left in the autumn of the year of their capture (Smith and Saunders, 1955). The annual average number of maturing eels taken in these ways from 1949 to 1954 was 697 (515–978). For 9 years, 1955–63, when the exclusion of young would be expected to have had an effect upon the number in the lake, the annual average was 321 (171–527). Difference in the averages is highly significant ($p < 0.01$). However, it is apparent that the attempt to rid the lake of eels by barring entrance of the young was only partially successful. Elvers were observed to move overland around the barrier on rainy nights. Some of the highest returns of planted brook trout to anglers coincided with the smaller numbers of maturing eels, by inference the smaller numbers of eels in the lake, but a possible causal relationship was obscured by the favourable effects of fertilization and other predator-control measures. It was not established whether fertilization and control of fish-eating birds and mammals affected the growth and survival of eels in the lake.

A gauge of the abundance of killifish was the number and size of schools that characteristically formed over littoral areas in summer. On this basis, killifish were observed to reach peaks of abundance 2 or 3 years after each fertilization. Predator control may have had an effect on their numbers, but after the first fertilization (1946) they became abundant before predator control was begun. Analyses of predator stomachs, including brook and rainbow trout, showed that killifish were eaten, but by no means commensurately with their numbers in the lake. Killifish are largely bottom feeders (Smith, 1947). In Crecy Lake they may have been a detriment to trout as competitors for a common food supply, rather than an asset as a forage fish.

CONCLUSIONS

Predator Control and Fertilization

Control of predators markedly improved anglers' catches of trout, particularly brook trout, planted in Crecy Lake, presumably through increased survival. Seining, gillnetting, scuba diving, observations of numbers of trout at spawning, as well as the general angling experience, showed that the catches

of trout reflected the numbers in the lake from year to year. Less apparent was a favourable effect of fertilization on yields of trout to anglers. Fertilization increased the trout food supply (bottom fauna) (Smith, 1961). Notable was the improved growth rate of planted brook trout underyearlings after fertilization, so that large numbers when yearlings were of an acceptable size to the anglers (Smith, 1955a). Without this situation of capture during the year after planting, the percentages of planted trout taken by anglers when predators were controlled would probably have been lower. Opening of the angling season at a later date in 1956 was followed by a lower percentage return to anglers of the underyearling brook trout planted in the autumn of 1955 (Smith, 1960). The result suggests strongly that natural mortalities not affected by the predator control applied, such as cannibalism, reduced the number of these fish in the lake before they were subjected to a deferred angling pressure. Highest yields of brook trout were realized when stocking, fertilization, and predator control were carried out together, rather than singly. Stocking presumably provided fish able to capitalize on an improved growth from fertilization and better survival of the faster-growing fish by predator control.

Brook or Rainbow Trout?

Yields of brook and rainbow trout to anglers at Crecy Lake were compared at similar rates of stocking, fertilization, and predator control. The data show that rainbow trout grew faster, but that this advantage in utilizing the lake's productive capacity was offset, in stocking with underyearlings, by an erratic and for the most part a lower percentage return to anglers. Plantings of brook and rainbow trout yearlings of attainable size gave similar yields. Since a majority of these fish were angled early in the year after planting, before appreciable growth could be made in the lake, such plantings were largely on a put-and-take basis.

The nonindigenous rainbow trout was in general inferior to the indigenous brook trout in providing angling in Crecy Lake when that lake was fertilized and when predator control measures were exercised. This was particularly the case in planting underyearlings when an objective was to use the improved productive capacity of the lake most fully.

ACKNOWLEDGMENTS

Mr G. Curtis Lowery efficiently maintained the field operations throughout the study. His competence as a naturalist was extremely helpful. Miss Mary Holmes competently assisted in filing and analysing data during the investigation. The assistance of Mr J. W. Saunders in many ways is also gratefully acknowledged. The author is indebted to Mr J. K. Lindsey for making the regression analyses by programming the IBM 1620 computer at the University of New Brunswick. The Resource Development Service, Department of Fisheries of Canada, provided the trout planted in Crecy Lake and also the services of Mr Lowery.

REFERENCES

BALDWIN, N. S. 1957. Food consumption and growth of brook trout at different temperatures. Trans. Am. Fish. Soc., **86**: 323–328.

BURDICK, G. E., M. LIPSCHUETZ, H. J. DEAN, AND E. J. HARRIS. 1954. Lethal oxygen concentrations for trout and smallmouth bass. N.Y. Fish Game J., **1**(1): 84–97.

BURDICK, MILTON E., AND EDWIN L. COOPER. 1956. Growth rate, survival, and harvest of fingerling rainbow trout planted in Weber Lake, Wisconsin. J. Wildlife Management, **20**(3): 233–239.

EIPPER, A. W. 1964. Growth, mortality rates and standings crops of trout in New York farm ponds. Cornell Agr. Exptl. Sta., Mem. 388. 67 p.

GRAHAM, J. M. 1949. Some effects of temperature and oxygen pressure on the metabolism and activity of the speckled trout, *Salvelinus fontinalis*. Canadian J. Res., D, **27**: 270–288.

HOAR, WILLIAM S. 1942. Diurnal variations in feeding activity of young salmon and trout. J. Fish. Res. Bd. Canada, **6**(1): 90–101.

MCAFEE, W. R. 1966. Rainbow trout. *In* Alex Calhoun [ed.] Inland fisheries management. State of Calif. Resources Agency, Dept. Fish Game. p. 192–215.

SHEPARD, M. P. 1955. Resistance and tolerance of young speckled trout (*Salvelinus fontinalis*) to oxygen lack, with special reference to low oxygen acclimation. J. Fish. Res. Bd. Canada, **12**(3): 387–446.

SMITH, M. W. 1947. Food of killifish and white perch in relation to supply. Ibid., **7**(1): 22–34.

1948a. Yield of speckled trout to anglers from certain lakes in New Brunswick and Nova Scotia. Fish. Res. Bd. Canada, Atlantic Progr. Rept. No. 42. p. 7–10.

1948b. Preliminary observations upon the fertilization of Crecy Lake, New Brunswick. Trans. Am. Fish. Soc., **75**: 165–174.

1952. Limnology and trout angling in Charlotte County lakes, New Brunswick. J. Fish. Res. Bd. Canada, **8**(6): 381–452.

1955a. Fertilization and predator control to improve trout angling in natural lakes. Ibid., **12**(2): 210–237.

1955b. Control of eels in a lake by preventing the entrance of the young. Canadian Fish Culturist, No. 17. p. 13–17.

1956. Further improvement in trout angling at Crecy Lake, New Brunswick, with predator control extended to large trout. Ibid., No. 19. p. 13–16.

1960. An attempt to improve trout fishing in a New Brunswick lake by deferment of opening of angling season. J. Fish. Res. Bd. Canada, **17**(5): 677–685.

1961. Bottom fauna in a fertilized natural lake and its utilization by trout (*Salvelinus fontinalis*) as food. Verh. Intern. Ver. Limnol., **14**: 722–726.

1966. Amount of organic matter lost to a lake by migration of eels. J. Fish. Res. Bd. Canada, **23**(11): 1799–1801.

1967a. Movement of planted hatchery-reared trout from a natural lake. Canadian Fish Culturist, No. 39. p. 35–40.

1967b. Observations of fish-eating birds and mammals at Crecy Lake, New Brunswick, over a 12-year period. Ibid., No. 39. p. 41–46.

SMITH, M. W., AND J. W. SAUNDERS. 1955. The American eel in certain fresh waters of the Maritime Provinces of Canada. J. Fish. Res. Bd. Canada, **12**(2): 238–269.

WALES, J. H., AND D. P. BORGESON. 1961. Castle Lake investigation — third phase: rainbow trout. Calif. Fish Game, **47**(4): 399–414.

Algal Growth and Primary Productivity in a Thermal Stream[1]

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ABSTRACT

Estimates of primary production and respiration of cyanophycean algal species growing in the run-off stream of a hot spring (37 C) were obtained throughout 1966 using the diurnal oxygen curve technique. Rates of gross primary production were slightly higher than published values from other aquatic ecosystems, but were less than recent estimates from hot springs in Yellowstone and Mount Lassen National Parks using the C_{14} uptake method. Production/respiration ratios were calculated and ranged from 1.1 to 5.0. The effect of photosynthesis on the ionic concentration of influent and effluent water was noted, together with morphological changes in the algal mat as the growing season progressed. Changes in algal standing stock were studied in 1964 and 1965, together with growth rates from denuded areas by two methods of harvest. In both years the most rapid growth was recorded in May and June, whereas during the winter months growth was negligible. Maximum values of primary production in May and June 1966 coincided with these maximum growth periods. Significant differences in growth rates between years were thought to be due to several factors; most notably, harvest methods and auto-inhibitory toxins produced by layers of senescent cells composing the "mature" algal mat. Energy losses due to algal export and grazing are discussed in relation to the dynamics of the mat, and an annual energy budget is derived from estimates obtained in 1966.

INTRODUCTION

Much is known of the primary productivity of phytoplankton in lakes, estuaries, and oceanic watermasses, but only recently have techniques been developed to allow reliable estimates of algal primary productivity in flowing waters. Little information is available about the primary production of thermophilic algae despite these recent advances in methodology, e.g. C_{14} uptake (Steemann-Nielsen, 1952; Brock and Brock, 1967) and the diurnal oxygen curve technique (Odum, 1956).

In recent years there has been a resurgence of attempts by aquatic ecologists to unravel the perplexing questions of growth, production, distribution, and "upper temperature limit" of thermophilic algae. Brock (1967) and Lenn (MS, 1966), using similar methods, have studied primary productivity along thermal gradients in hot springs in Yellowstone and Mount Lassen National Parks, USA, during the summer months, and Castenholz (1967) has comprehensively reviewed the environmental requirements of thermophilic algae.

However, to date no attempts have been made to ascertain in detail the seasonal cycle of primary production, algal growth, and standing stock in a

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hot spring at temperate latitudes using the diurnal oxygen curve technique. Unfortunately, past studies have been relatively short term and have therefore failed to relate monthly summer observations to long-term seasonal or annual changes. The relative constancy of the thermal stream environment, which simulates a "laboratory in the field" situation, afforded an excellent opportunity to estimate monthly, seasonal, and yearly changes in algal standing stock, growth, and production.

CHEMISTRY, GEOLOGY, AND A DESCRIPTION OF THE STUDY AREA

The Ohanapecosh Hot Springs (elevation 612 m) lie in a valley in the southeast corner of Mount Rainier National Park, Washington State, USA, (46°44′N, 121°34′W). These nine springs are typical examples of alkaline, calcareous springs, deriving most, if not all, of their water from ground water which leaches various cations from the parent crystallized igneous rock as it ascends the fissure (D. Mullineaux, personal communication). This dissolution results in the formation of bicarbonates, especially with sodium and potassium, and the water is made slightly alkaline. These springs deposit travertine ($CaCO_3$), and banks of travertine attain a depth of 7 m in certain areas, testifying to the age of the thermal activity in this region. The temperature of the springs ranges from 35 to 48 C but there is little variation in their water chemistry. A partial chemical analysis of the water from the main study spring for this particular investigation, spring 3, together with a cation–anion balance sheet, appear in Table I.

In April 1963, a wooden trough, approximately 3 m × 12 cm, was permanently inserted into this spring (see map, Fig. 1) to provide an artificial stream with known dimensions (3146 cm²) and flow rate (4.8 liters/min). The temperature of the water as it entered the trough was 37 C with no measurable variation. The temperature recorded at the end of the trough was

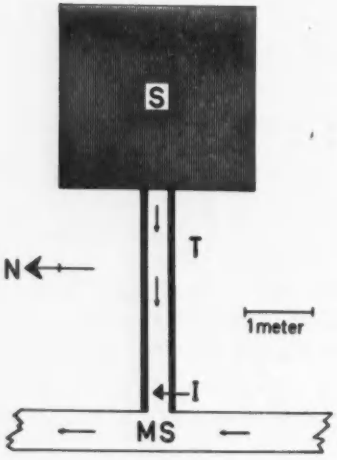

FIG. 1. Main study area, spring 3. S = capped spring source covered with black polyethylene; T = experimental trough; I = site for physical and chemical readings; MS = main run-off stream; ↑ = direction of water flow.

more variable, 36 ± 2.2 C, depending upon ambient temperature and illumination. The blue–green algal species *Schizothrix calcicola* Gom. and *Phormidium* sp.[3] rapidly colonized the surface of the trough, and this substrate was then sampled throughout the 4-year study period. All results presented and discussed were derived from samples taken from this source, but supplemental observations were obtained from similar algal mats on natural substrates in other run-off streams within the general study area.

MATERIALS AND METHODS

Throughout the winter months, from November to April, monthly sampling trips were made, and during the summer, biweekly trips were taken, often lasting 5 or more days. In 1966 I was able to spend most of the summer at the study site, uninterrupted. The following procedures were employed for data collection and analysis.

Temperature

Temperature measurements were obtained at the spring pool (center of or above source), at the outlet of the run-off stream, and at predetermined intervals along the run-off stream. When collections of flora and fauna were made, the temperature was measured and recorded at the precise location of collection. In 1966 temperature measurements obtained with standard Centigrade thermometers were supplemented with recordings taken with a Fenwal thermistor, No. GB32p8. Short-term, 1-min interval temperature changes in the thermal stream during the day were recorded during O_2 measurement in spring 3 in 1966.

Radiation

Total sun plus sky radiation was measured with a Belfort, series No. 659, recording solarimeter. This instrument was calibrated with a recording pyrohelimeter operated by the Department of Atmospheric Sciences, University of Washington, Seattle, Wash. An RCA, No. 4413, cadmium sulfide photoconductive cell was used to record light changes at spring 3 during operation of the instrument designed to record diurnal oxygen curves (Fig. 2).

Oxygen

Several methods were used to measure the amount of dissolved oxygen in the spring water. Methods that proved most amenable were the standard Winkler method (American Public Health Association, 1965, p. 307) with the Pomeroy–Kirschman alkali reagent (Carpenter, 1965), and an oxygen electrode (Fig. 3) designed by Dr John Kanwisher, modified from the original design (Carritt and Kanwisher, 1959). The Winkler method was used during 1964 and 1965 sampling periods and the electrode during fall of 1965 and throughout 1966. The electrode was not used in water at a temperature above

[3]Identification by Dr F. Drouet, Academy of Natural Sciences, Philadelphia, Pennsylvania.

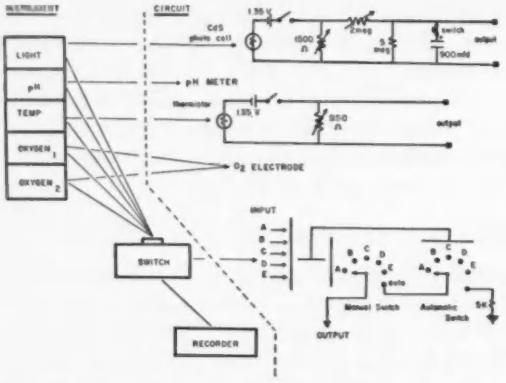

FIG. 2. Basic design of instrument used in 1966 to measure oxygen, *p*H, light, and temperature in the field. Circuits for light integrator, thermistor probe, and automatic switching device included.

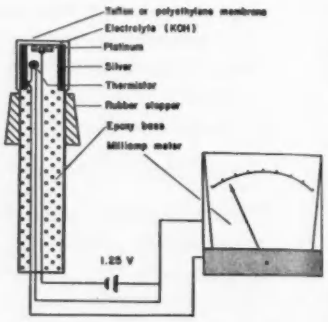

FIG. 3. Simplified circuit diagram of oxygen probe used in this investigation.

40 C. It was calibrated to zero in the laboratory with pure nitrogen, and in the field with Na_2SO_3 solution. Full scale, 100 per cent saturation, was obtained by letting the electrode equilibrate for approximately 1 hr in the atmosphere. When in use the electrode was periodically checked by Winkler determinations, and the agreement between sampling periods was consistent. A new membrane, either teflon or polyethylene, was put on after 24 hr of continuous use, and the electrode recalibrated.

*p*H

The hydrogen ion concentration was determined with a portable battery-operated Beckman *p*H meter, Model G, with an accuracy of ± 0.02 *p*H units. Measurements obtained in 1966 were taken with a line-operated Leeds & Northrup *p*H indicator, Model 7405, with an accuracy of 0.02 *p*H units.

Diurnal Oxygen Curves

An instrument was designed by Dr H. S. Horn, Princeton University, to simultaneously record input from two Kanwisher electrodes, *p*H meter, thermistor, and a cadmium sulfide light cell (Fig. 2). Input signals were automatically switched to a Heathbuilt servo-recorder, Model No. EUW-20A. Two heavy-duty 12-v storage batteries were used with a Terado power converter, Model No. 50134. This system provided a convenient, portable source of a-c current of appropriate specifications to operate the equipment for about 8 hr before the battery had to be changed and recharged. A small section of a field record is reproduced (Fig. 4) to show the type of output received from the recorder and automatic switching device. Order of input could be arranged arbitrarily to suit the desired experimental design.

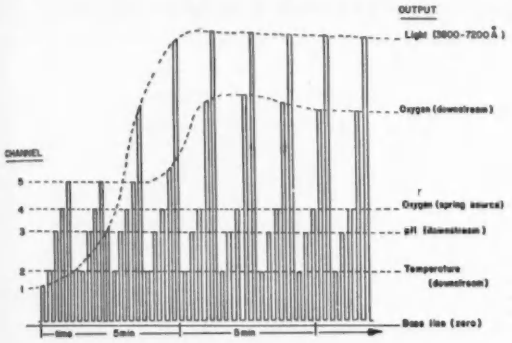

FIG. 4. Duplicate of actual field record obtained August 4, 1966, from instruments described in text and shown in Fig. 2.

Since the spring source, covered completely with heavy (folded thickness, 4 mil) black polyethylene, acted like a chemostatically regulated inflow with no diurnal variation, the instrument was set at predetermined distances from the source to obtain diurnal oxygen, *p*H, temperature, and radiation curves. Operation of the instrument throughout the night provided a measure of algal respiration. Experiments were conducted using heavy sheets of black and clear polyethylene to measure changes in diurnal curves brought about by shading portions of the stream and by covering it completely.

CHEMICAL ANALYSES

In addition to the partial ion analysis (Table I), total phosphorus, phosphate, nitrate, total dissolved solids, and alkalinity were determined periodically throughout the study period (Table II). Determinations of total phosphorus were made according to methods established by Hansen and Robinson (1953), and methods of Zwicker and Robinson (1944) were used for photometric determination of nitrate. Phosphate was determined using methods of Robinson and Thompson (1948). Alkalinity determinations followed procedures outlined in American Public Health Association, (1965, p. 44). Dichromate oxidation (C.O.D.) methods (American Public Health Association, p. 399) were used to estimate dissolved organic matter.

An EEL Atomic Absorption Spectrophotometer,[4] Model No. 140, was employed to determine diurnal variation in Ca^{++} and Mg^{++}. A 0.05% solution of lanthanum chloride was added to each sample to reduce phosphate interference.

ALGAL PRIMARY PRODUCTION

Estimates of primary production and respiration involve amounts of O_2 exchanged with the aqueous environment, and before meaningful results can be obtained one must correct for the effects of diffusion. Estimation of

[4]Evans Electroselenium Ltd., St. Andrews Works, Halstead, Essex, England.

TABLE I. Summary of partial ion analysis, spring 3, September 1965.

Constituents	
*p*H (25 C, laboratory measurement)	7.3
Bicarbonate alkalinity, mg/liter, $CaCO_3$	813
Carbonate alkalinity, mg/liter, $CaCO_3$	0
Conductivity 25 C, μmho/cm	4,470
Iron, mg/liter Fe^{++}	0.11
Sodium, mg/liter Na^{+}	925
Potassium, mg/liter K^{+}	61
Calcium, mg/liter Ca^{++}	156
Magnesium, mg/liter Mg^{++}	3.8
Sulfate, mg/liter $SO_4^{=}$	160
Chloride, mg/liter Cl^{-}	820

Cation–anion balance. All values in milli-equivalents/liter.

Ion	Cations	Anions	
H^{+}	0.0	HCO^{-}_3	16.3
Fe^{++}	0.0	$CO_3^{=}$	0.0
Na^{+}	40.2	$SO_4^{=}$	3.3
K^{+}	1.2	Cl^{-}	23.1
Ca^{++}	7.8		
Mg^{++}	0.3		
Total	49.5		42.7

TABLE II. Chemical analyses, spring 3.

Date	NO_3–N (*μg/liter*)	Total P (*μg/liter*)	PO_4–P (*μg/liter*)	Alkalinity[a] (*mg/liter*)	T.D.S.[b] (*g/liter*)
May 9, 1963	48	92	53	834	2.76
Sept. 12, 1964	37	91	61	821	2.68
June 10, 1965	–	71	–	831	2.62
Aug. 20, 1966	35	68	42	822	2.60
May 1, 1967	62	95	48	817	2.67
June 30, 1967	64	74[c]	41	824	2.63

[a]Bicarbonate as $CaCO_3$.
[b]Total dissolved solids.
[c]Soluble 64, particulate 10.

net amounts of oxygen evolved (uncorrected for diffusion) requires measurement of amounts of oxygen in influent and effluent water. Since the amounts in influent water (spring source) remained constant throughout the day and night there was no need to continuously monitor it. The effluent measurements

were taken at the end of the trough every minute and recorded together with light and temperature on a servo-recorder (Fig. 2, 4).

These data, together with knowledge of exchange rate and volume of water in the stream, permitted the calculation of net oxygen change per unit time according to the following expression (modified from McIntire et al., 1964):

$$\text{Net } O_2 \text{ change (mg } O_2/m^2 \text{ per hr)} = Ft\left[\frac{E_0 + E_1}{2} - I\right] + V(E_1 - E_0)$$

where F = exchange rate in liters/hour; t = time in hours; E_0 = dissolved O_2 concentration in mg/liter of effluent water at beginning of time interval; E_1 = dissolved O_2 concentration in mg/liter of effluent water at end of time interval; I = dissolved O_2 concentration in mg/liter of influent water; V = volume of water in trough in liters.

To estimate gross production, measurements of respiration were obtained by placing a black sheet of polyethylene over the trough or by allowing the recording apparatus to run through the night, and oxygen change was calculated as outlined above. The rate of gross production per unit time was obtained by summing net production and respiration values after both were corrected for diffusion of oxygen at the water surface.

In the field, rates of diffusion into the water flowing in the trough without any algae present were established. In addition to these measurements, the stream could easily be manipulated to distinguish effects of diffusion, production, and respiration by using sheets of both black and clear polyethylene. When the polyethylene was placed in a domelike fashion over the stream, air could easily diffuse into the water, but light could not reach the algal surface since the dome of polyethylene extended well beyond the end of the trough. Therefore, changes in oxygen were caused both by respiration and diffusion when black sheets were used, whereas clear sheets provided estimates of net production and diffusion. When sheets were placed on the stream to form an effective air-tight seal, then the black polyethylene covering gave reasonable estimates of respiration, and the clear sheet gave net production. Thus the effects on the observed oxygen changes of these aforementioned factors could be readily assessed. Since the dominant insect herbivores, *Hedriodiscus truquii* Bellardi and *Caloparyphus* sp., utilized atmospheric oxygen, the respiration rates given should not be confused with community rates often obtained in a similar fashion (Odum, 1956; McIntire et al., 1964), but should be interpreted as respiration estimates of the algae and associated bacteria.

Standing Stock and Algal Growth

Samples of *Schizothrix calcicola* and *Phormidium* sp. mat growing in the trough were sampled at biweekly intervals commencing in 1964. The mat was scraped from the trough with a spatula at each sampling period during

1964 (Fig. 5), and portions of it brought back to the laboratory for quantitative evaluation and caloric determination. Samples were dried in an oven at 60 C, weighed, ground to a fine powder, and burned in a Parr Semimicro Oxygen Calorimeter, Model No. 1411, to determine calories/gram of dry weight and ash-free dry weight. Portions of the powdered sample were ashed for 2–3 hr at 500 C. Caloric values were corrected for endothermy due to the considerable ash content ($CaCO_3$) per sample using corrections discussed by Paine (1966).

The experimental design was modified in 1965 (Fig. 5) to allow the algal mat to build up in a normal fashion as the growing season progressed. Portions

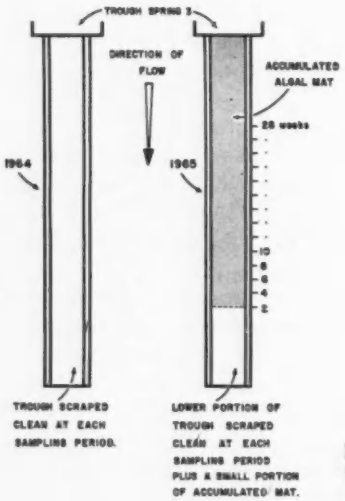

FIG. 5. Summary of sampling procedures used for the estimation of net growth and biomass in 1964 and 1965.

of this accumulated algal mat growing in the lower sections of the trough were sampled at 2-week intervals (2, 4, 6, ... to 28 weeks). In addition to these data, algal growth which developed directly below the accumulated mat in the area denuded by previous sampling (Fig. 5) was scraped clean at biweekly intervals. All samples obtained in 1965 were analyzed similarly to those previously described. Thus an evaluation of growth rate at 2-week intervals vs. growth of accumulated mat in spring 3 could be assessed and directly compared with 1964 values obtained by complete denudation at each sampling period.

Each sample obtained from the wooden trough in 1964 was compared with similar samples of algal mat taken from natural substrates with respect to general appearance, ash, and organic content, and no obvious differences could be ascertained.

Each sample collected during 1964 and 1965, with the exception of accumulated mat samples in 1965, consisted of eight separate 2-cm^2 quadrats taken at random from the surface of the trough. A mean and variance were

calculated for each sample, and mean values were used in the compilation of figures and tables. It was assumed that with an independent mean and variance, these data of biomass were normally distributed, and thus the *t*-test, regression analysis, and analysis of covariance could be used as appropriate test statistics, without transformation, for the objective analysis of these data.

It should be noted that throughout the following discussion, results are expressed in terms of grams organic matter which is equivalent to loss on ignition (500 C).

RESULTS AND DISCUSSION

Algal Primary Production

Primary production estimates were obtained from both diel and diurnal oxygen changes, measured from 1966 onwards from the trough in spring 3. The algal mat was undisturbed throughout the year and allowed to accumulate in a normal manner. Before estimates of gross primary productivity could be obtained, diel changes in oxygen concentration had to be corrected for diffusion.

Mean values for diffusion of oxygen into the water ranged from 470 mg O_2/m^2 per hr in August to 660 mg O_2/m^2 per hr in April. The mean rate of diffusion when no algae were present, i.e. trough denuded, was 752 mg O_2/m^2 per hr. The oxygen concentration of the spring water, as it entered the trough at a temperature of 37 C, was a consistent 3.85 mg O_2/liter or 57% saturated with oxygen, and on only one occasion did the oxygen concentration exceed 100% saturation. This followed direct and sudden exposure to full sunlight after removal of the black polyethylene covering from the trough. Estimates of diffusion obtained by covering the stream with black polyethylene accompanied every diel oxygen determination.

Gross primary production in spring 3 ranged from 5.8 to 11.3 g O_2/m^2 per day during the summer and algal respiration from 1.9 to 2.7 g O_2/m^2 per day (Table III). Photosynthesis/respiration ratios never fell below 1.0, ranging from 1.59 in December to 5.04 in May. Lenn (MS, 1966), using carbon 14 assimilation to estimate primary production of Cyanophyta in Drakesbad Hot Springs, Mount Lassen National Park, California, obtained summer values that ranged from 7 to 12 g C/m^2 per day. To compare these values expressed as grams C/m^2 per day requires conversion of carbon uptake to oxygen liberation. If the photosynthetic quotient (P.Q.) were 1.0, ($\frac{CO_2}{-O_2}$ = 1.0, molar basis), carbon values would be multiplied by 2.76 (weight basis, mg/liter) to give oxygen. More realistically, however, P.Q. is less than 1.0. According to Winberg (1961), 0.8 is the best value to use for plankton, giving a factor of 3.76. Thus, the values obtained from spring 3, ranging from 4.2 to 2.2 g C/m^2 per day during the summer, appear to be consistently lower than Lenn's values of primary production. This discrepancy is not surprising

TABLE III. Primary production, spring 3, 1966 (values in g O_2/m^2 per day)

Date	Gross production	Respiration	Net production	P/R ratio
Mar. 23	2.91	0.99	1.91	2.91
Apr. 8	4.28	1.00	3.27	4.26
Apr. 30	7.26	1.60	5.65	4.52
May 27	10.14	2.01	8.13	5.04
June 28	11.34	2.70	8.64	4.20
July 20	9.36	2.69	6.67	3.48
Aug. 2	8.05	2.44	5.61	3.29
Aug. 8	7.97	2.51	5.45	3.17
Aug. 20	5.88	1.98	3.90	2.97
Sept. 10	4.53	1.47	3.06	3.09
Oct. 9	5.38	1.76	3.62	3.05
Oct. 21	2.89	0.85	2.04	3.41
Oct. 24	2.61	0.72	1.89	3.63
Dec. 14	0.89	0.56	0.33	1.59

since Lenn was working with a different algal assemblage, i.e. *Synechococcus* spp., in a thermal gradient 15–20 degrees C higher, and also in a spring whose water chemistry differed considerably from spring 3.

An estimate of the amount of organic matter equivalent to 1355 g O_2/m^2 per year (362 g C/m^2 per year) was obtained by integration. Net production was 1150 g O_2/m^2 per year (306 g C/m^2 per year) and respiration 205 g O_2/m^2 per year (56 g C/m^2 per year). It is most informative to compare daily production values with Westlake's (1965) tabulation of published results from other aquatic environments. Values from spring 3 are substantially higher than results from productive eutrophic lakes during optimum conditions in temperate latitudes (0.19–1.2 g C/m^2 per day), but very similar to production by aquatic macrophytic vegetation during the summer in lakes and rivers (1.5–4.2 g C/m^2 per day). Production estimates by McIntire et al. (1964) on periphyton in artifical streams were much less (2.4–4.7 g O_2/m^2 per day) than values from spring 3 during similar time periods.

Algal production in spring 3 is obviously correlated with incident radiation (Table III), diminishing in late fall, remaining negligible until March, and increasing again with increasing light intensity. A plot of gross production as a function of light intensity showed a linear relationship (Fig. 6), with a correlation coefficient of $r = 0.842$.

The photosynthetic response of algae in spring 3 to sudden change in illumination was very rapid, as indicated by oxygen change. For example, on August 20, a bright, clear day, the recorded oxygen content at the end of the trough with the stream totally exposed to full sunlight was 5.30 mg/liter. When the stream was loosely covered (diffusion occurring) with black polyethylene, in less than 4 min the recorded oxygen concentration was 4.26 mg/liter. When the stream was again uncovered and fully exposed to sunlight,

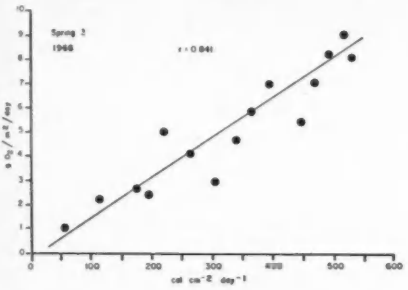

FIG. 6. The regression of grams O_2/m^2 per day to light intensity with correlation coefficient as shown in figure.

within 3 min the value rose to 5.28 mg/liter which represents an uptake of 0.70 mg O_2/liter per min or 14 mg O_2/m^2 per min over the 3-min period. This same rapid response to light changes was evident when the first full sunlight hit the algal mats in the morning. A similar rapid response in oxygen concentration concomitant with sudden light changes was noted by M. Pamatmat (personal communication) in his flow chamber that enclosed benthic communities in the intertidal zone, False Bay, Washington State.

Another interesting feature of algae in all springs was the striking color variation exhibited seasonally. In early spring and late fall a dark green color (chlorophyll predominance) is very evident whereas in midsummer it is a light green to pale yellow color (carotenoid predominance). Though not adequately tested, this is undoubtedly the manifestations of the phenomenon-complementary chromatic adaptation. Such changes provide optimal photosynthetic efficiency throughout a range of light intensity. This process of pigment complementation is well known and has been discussed relative to the thermophilic Cyanophyta by Castenholz (1967).

Cursory examination of the effects of algal photosynthesis on ionic concentrations of influent and effluent water after it has passed over the algal mat yielded interesting results which merit brief discussion and further investigation. On a clear day in June, analysis of NO_3–N, total phosphorus, dissolved organic matter (C.O.D.), and total dissolved solids (T.D.S.) showed significant (t-test, $p = 0.05$) reduction of NO_3–N between upstream–downstream values indicating a mean uptake of 215 $\mu g/m^2$ per min (Table IV),

TABLE IV. Water chemistry, spring 3, June 29, 1967.

Analysis	Determinations	Source (*mean value*)	End of trough (*mean value*)	Uptake ($\mu g/m^2$ *per min*)
NO^3–N (*μg/liter*)	6	68	54	215
Total P (*μg/liter*)	6	74	68	92
Dissolved organic matter (C.O.D.) (*mg/liter*)	8	22.6	22.6	–
Total dissolved solids (T.D.S.) (*g/liter*)	4	2.64	2.61	–

and though not statistically significant ($p = 0.05$), there was a suggestion of an uptake of phosphorus (92 $\mu g/m^2$ per min) as the water passed over the mat. Though nitrogen fixation has been reported from mats of thermophilic Cyanophyta (Dugdale, 1967), it is apparently not occurring within the *Schizochrix calcicola*, *Phormidium* sp. mat. This of course does not exclude the possibility that, at other times and under a different set of environmental conditions, these species may not be involved in nitrogen fixation. There was no discernible variation between upstream–downstream values of T.D.S. and C.O.D.

Another experiment involved the determination of Ca^{++} and Mg^{++} concentrations concomitant with diurnal changes in net primary productivity (Table V). There seems an obvious inverse correlation between net production

TABLE V. Diurnal variation in net primary production and calcium and magnesium concentrations, spring 3, September 10 and 11, 1966.

Time interval	Net primary production (*mg O_2/m^2 per hr*)	Time water sample taken	Mean Ca^{++} (*mg/liter*)	Mean Mg^{++} (*mg/liter*)
Sept. 10				
0800–0900	26	0900	61.2	5.0
1230–1330	97	1335	37.7	5.0
1430–1530	94	1520	15.6	4.8
1730–1830	52	1830	22.1	5.0
1830–1930	31	1945	20.7	4.8
Sept. 11				
0800–0900	22	0845	25.0	4.9

and Ca^{++} concentration in water flowing over the mat, but little variability in Mg^{++} ion concentration. A probable partial explanation for this variability of Ca^{++} with increasing production is the withdrawal of free CO_2 from solution by actively photosynthesizing algae with the resultant precipitation of $CaCO_3$ within and around the gelatinous matrix surrounding the filaments, forming layers or "lamellae" of $CaCO_3$ that are so conspicuous in thermophilic Cyanophyta mats at Ohanapecosh (Fig. 7).

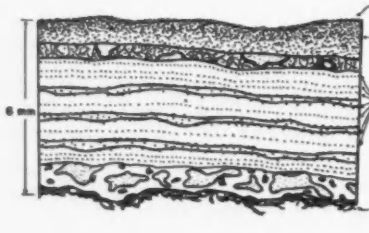

FIG. 7. Simplified sketch of a "typical" thermophilic Cyanophyta mat from Ohanapecosh Hot Springs showing layering with associated $CaCO_3$ crystals. The mat was sliced into thin cross-sections and observed under 100× magnification.

Estimates of primary production presented and discussed here are probably low due to direct loss of O_2 as bubbles to the atmosphere immediately after full sunlight reaches the algal mat, and reliability of these data will of course depend upon the accuracy of diffusion estimates. The C_{14} method recently discussed by Brock and Brock (1967) was not attempted at the study site since it required that a small amount of algae be removed from the stream and placed in a vial for a given period of time, usually over an hour. It has been shown by several investigators, most notably by Whitford (1960), that production in streams may be directly related to current velocity as well as light, and therefore results of C_{14} assimilation of the algae in standing water may be open to some question since the in situ effect of current is essentially ignored. Changes in the diurnal *p*H curve measured at spring 3 were slight and reliable calculations of production based on *p*H variation could not be made since the spring was charged with free CO_2 (Beyers and Odum, 1959). Thus, the diurnal oxygen curve seemed the best method available at the time for the estimation of primary production in the thermal run-off stream.

As more information comes forth it is evident that the thermal stream is one of the most productive of all aquatic habitats, with values ranging from 4 to 12 g C/m² per day during optimum conditions. One cannot help but be impressed by the striking similarity between the community of grossly polluted aquatic environments and thermal streams. Besides the obvious similarity of simplicity or lack of diversity, and the generally harsh abiotic environment, a very high rate of organic próduction appears as a characteristic common to both environments.

Biomass and Algal Growth Rates

The seasonal variation in biomass and growth rates was estimated from algal material scraped from the trough extending from the spring source. The term "growth" as used throughout the following discussion refers to the net rate of accumulation of organic matter per unit time (T), and can be defined by the following expression: net growth rate = accumulated organic matter/T = gross production–respiration–grazing–export/T. It should be borne in mind that T in the above expression usually refers to a 2-week period and should not be confused with instantaneous rates.

When reference is made to the accumulated algal mat samples in 1965, the most suitable term is "accumulated biomass" rather than growth, for, as the algal mat continues to grow throughout the season, growth diminishes and other factors such as respiration, grazing, and export begin to play an increasingly important role in the dynamics of the mat.

1964 Harvest

The maximum growth attained between sampling intervals, estimated by complete denudation of the trough, occurred from May 18 to June 5, and was 8.6 g organic matter/m² per 17-day growth period. This value represents an average growth rate of 505 mg organic matter/m² per day (Table VI). The

TABLE VI. Growth and biomass determinations, spring 3, 1964–65.

Time interval	Total accumulation per period (*g organic/m²*)	Mean growth rate (*mg organic/m² per day*)	Accumulated biomass (*g organic/m²*)
1964			
Feb. 20–Mar. 15.	0.29	12	–
Mar. 15–Apr. 3	0.98	52	–
Apr. 3–May 18	1.62	114	–
May 18–June 5	8.60	505	–
June 5–21	4.45	261	–
June 21–July 6	3.86	257	–
July 6–20	0.98	70	–
July 20–Aug. 7	2.00	111	–
Aug. 7–21	0.73	52	–
Aug. 21–Sept. 7	0.77	45	–
Sept. 7–25	0.61	34	–
Sept. 25–Oct. 10	0.17	11	–
Oct. 10–Nov. 7	0.15	5	–
Total	25.21		
1965			
Mar. 1–21	0.50	25	–
Mar. 21–Apr. 10	1.76	88	–
Apr. 10–24	1.75	125	1.90
Apr. 24–May 8	0.68	49	2.50
May 8–22	1.08	78	3.12
May 22–June 5	1.54	110	3.92
June 5–19	1.83	131	4.75
June 19–July 3	0.94	67	5.96
July 3–17	0.99	71	5.00
July 17–Aug. 1	0.64	46	4.16
Aug. 1–15	0.47	34	5.61
Aug. 15–30	0.68	49	5.69
Aug. 30–Sept. 14	0.76	54	5.00
Sept. 14–28	0.33	24	6.89
Sept. 28–Oct. 12	0.36	26	6.10
Oct. 12–26	0.27	19	7.40
Oct. 26–Nov. 12	0.11	7	7.64[a]
Total	14.69		

[a]Total accumulation in 257 days.

lowest values were obtained in early spring and late fall over the periods from February 20 to March 15 (0.29 g organic matter/m² per 24-day growth period or an average growth rate of 12 mg organic matter/m² per day) and from October 9 to November 7 (0.16 g organic matter/m² per 30-day growth period or 5 mg organic matter/m² per day). The annual monthly mean growth pattern tended to parallel the sun plus sky radiation curve for this year (Fig. 8).

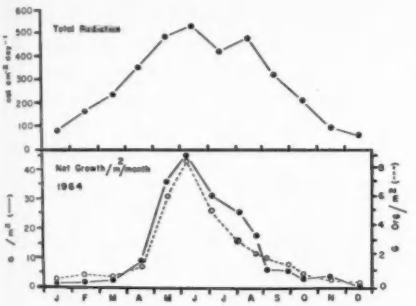

FIG. 8. Total radiation and net growth of algae in grams dry weight and grams organic matter (less ash)/m² per month. Values represent monthly means, 1964.

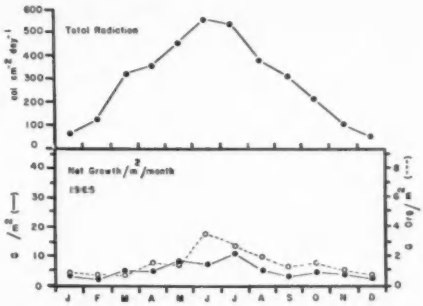

FIG. 9. Total radiation and net growth of algae in grams dry weight and grams organic matter (less ash)/m² per month. Values represent monthly means, 1965.

1965 HARVEST

Only the lower portion of the trough was denuded, as in 1964, at each sampling interval, and the rest of the algae in the upper portion of the trough was allowed to accumulate undisturbed except for small sections sampled at 2-week intervals to measure the rate of accumulation of organic matter as the growing season progressed. This deviation in harvest method yielded very different results in estimates of algal growth rates between years. Comparison of Fig. 8 and 9 and values given in Table VI will serve to illustrate this difference in growth between years. With the exception of harvest methods already noted, data for the compilation of these figures and table were obtained in a similar manner and the values are therefore directly comparable. Further evidence of a significant decrease in growth rate in 1965 is shown by a plot of total radiation vs. net growth for the 2 years under comparison (Fig. 10). The slopes of these regression lines were compared using analysis of covariance, F test statistic, and they proved to be significantly different at the $p = 0.01$ level.

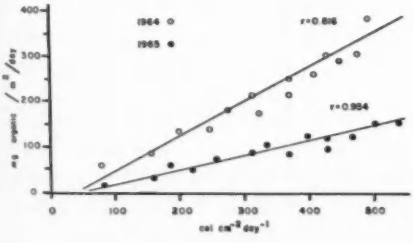

FIG. 10. Regression of net growth of algae in milligrams organic matter/m² per day in 1964 and 1965 to daily radiation with correlation coefficients as shown in figure. Slopes were compared using analysis of covariance, F-test statistic.

How can one explain or interpret this difference in growth between years? First, since measurements of radiation and water chemistry were nearly identical in both years, the difference is more likely attributable to variation in

sampling methods used to obtain these estimates. In 1965 water flowed from the covered spring source over and within the accumulated algal mat, and finally to the denuded lower portion of the trough where at 2-week intervals samples were taken. It seems obvious that water reaching algae in the lower portion of the trough in 1965 may have been altered, making conditions less suitable for growth. Unfortunately, chemical analysis of water in 1965 was done only on source water and therefore can provide no evidence of nutrient depletion. Evidence obtained from analyses done in 1967 and throughout the study period (Tables II and IV), suggests that nutrient depletion does not play a role in the observed difference in growth rates.

It is possible that the accumulation of algal or bacterial cellular products within the accumulated mat may in part account for this difference in growth between years (antibiotic effects). It is known that blue–green algae may liberate extracellular substances, often with deleterious effects on both plants and animals (Gorham, 1964; Lefevre, 1964). The toxicity is often associated with algal decomposition products and can vary widely in amount and subsequent effect. Examination of the morphological features of the accumulated algal mat lends credence to the hypothesis of a possible antibiotic effect.

Algal Mat Morphology

The thick algal mats display a characteristic morphology with conspicuous lamellae (bands of $CaCO_3$) evident in thinly sliced cross sections (Fig. 7). These lamellae are probably not annual layers since some of the mats begin anew each spring, usually having been destroyed in late fall by heavy rain and decomposition with subsequent sloughing. These layers may represent the effect of extended periods of clear weather with resultant maximum production, rapid growth, and consequent removal of CO_2 prompting the deposition of $CaCO_3$. During rapid growth, newly formed filaments would overgrow and shade out other filaments less capable of growth and migration to the well-lighted surface of the mat. This shading effect would then result in layers of filaments and associated bacteria in various stages of decomposition with actively growing filaments on top. This "layering" effect has also been observed by Brock (1967) who speculates that they probably represent annual layers. My own observations tend to refute this idea, but it is clear that further research is necessary before the cause(s) of these puzzling layers are fully understood.

It is this layer(s) of senescent cells that perhaps liberates extracellular products that may exert deleterious effects on algae growing in water directly below a "mature" accumulated mat. In 1964 the effects would have been minimal since the mat was completely removed at each sampling period, i.e. was not allowed to accumulate beyond a maximum of 30 days. If the mats liberate a substance, then interesting questions logically ensue that at present cannot be answered: (1) why does the mat not thin-out downstream during the growing season and (2) should not growth in upper portions of the thermal streams be noticeably higher than lower sections? I have never been

able to detect any significant difference in growth between upper and lower portions of the trough. In 1967 I sampled the trough specifically for this reason and no differences were found. If a liberated substance is acting as a deterrent to algal growth, it would be difficult to test in the field, and I have not tested for it.

Growth Characteristics of the Accumulated Mat, 1965

There are several features of interest that were noted as the undisturbed mat in the upper portion of the trough changed during the summer. The mat increased in thickness from about 1 mm in April to 1 cm in November, when the mat began to gradually slough and noticeable deterioration was evident. In December very little was left of it except accumulated ash (iron precipitate and $CaCO_3$) with a thin skin of algae on top. As the mat increased in thickness there was a steady increase in ash content, 69–85%, and a decrease in organic content, 31–15% on a dry weight basis (Fig. 11). This increase in ash is pro-

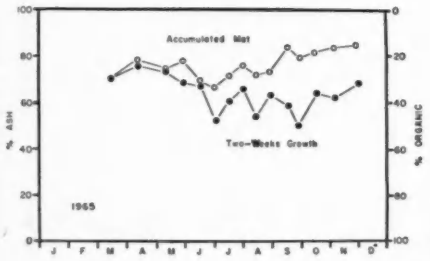

Fig. 11. Variation in percent ash and organic content between biweekly samples from the lower trough and accumulated mat during the growing season, 1965.

bably a consistent feature of all filamentous Cyanophyta mats in alkaline and calcareous hot springs when they colonize a new substrate and mature during the growing season. It would be most informative to compare these values with similar determinations from established algal mats in reasonably stable thermal gradients, as exist for example in Yellowstone National Park. The ash content of samples harvested at biweekly intervals did not deviate significantly from the mean value, 64%, and seasonal fluctuations could not be correlated with growth rates or similar parameters that may in one way or another be related to ash deposition. Similarly, the caloric values of these samples did not deviate significantly from the calculated mean for 31 determinations: 1264 cal/gram or 4543 cal/ash-free gram, SD 61. Unfortunately, caloric values of accumulated mat were not obtained and cannot, therefore, be compared with these 2-week values.

The organic content per m^2 of accumulated mat in 1965 increased steadily in the spring, leveled off during July, August, and September, and peaked at 7.6 g/m^2 in November after 257 days of growth (Table VI). The period of little change in midsummer undoubtedly reflects the biological interaction of

competition for light, space, and dissolved gases, that begins to play an increasingly important role as the mat matures. If one adds the average growth per 2-week period of algae sampled from the lower portion of the trough in 1965 (column 1, Table VI), and compares this total with the maximum figure for organic accumulation of undisturbed mat, 7.6 (column 3, Table VI), then this apparent difference, 7.0 g organic/m², should provide a rough approximation of losses from increased respiration, export, and grazing. This assumes of course that export and grazing are insignificant losses to algae growing in the lower trough, and that respiration is less relative to growth where harvesting is done at regular 2-week intervals. My own observations tend to validate these assumptions, at least for losses of export and grazing. Due to periodic removal, herbivores were seldom encountered in the lower trough, whereas the herbivores *Hedriodiscus truquii* and *Caloparyphus* sp. were often observed on the upper portion of accumulated mat. Losses by export were studied in some detail in 1966, and it was the mature mats that contributed the greatest proportion of material.

Algal Export

Throughout 1966, at monthly intervals, the amounts of algae exported were estimated from samples obtained from a 1-mm² mesh net placed in a constricted portion of the main run-off stream. This net filtered water draining an estimated 31 m² of algal mat surface. At predetermined intervals throughout the day and night, the net was cleaned and samples preserved with formalin. The samples were sorted into two fractions, invertebrates and algae, and any terrestrial import, i.e. leaves, pine needles and seeds, grasses, and insects, was removed before the samples were weighed and ashed.

The annual variation of algal export material is characterised by two periods of peak export, the major one occurring from April to August, bracketing the period of maximum production and growth, and the second period (not tested for significance) occurring from October to December (Fig. 12). The latter peak, which is minor when compared with summer values, is thought to represent an increase due to gradual decomposition and subsequent sloughing of the mats concomitant with decreasing light intensities.

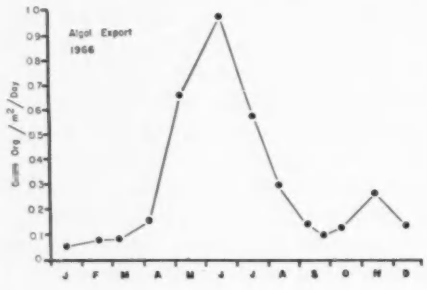

FIG. 12. Annual variation in algal export from an estimated 31 m² of mat surface. Lines fit to mean values.

A mechanism partially responsible for this observed pattern is suggested by consideration of diurnal patterns of export, recorded during the summer from samples taken at 2-hr intervals on August 4 and 5, 1966. These data were selected as examples because of the contrasting weather conditions existing during this 2-day period. August 4 was a cloudy day with scattered sunny periods, whereas August 5 was bright, clear, and cloudless. The difference in total organic export per day was striking, as was the apparent positive correlation of export per hour with total radiation measured during this period (Fig. 13). The mean export per hour on August 4, was 0.43 g organic matter

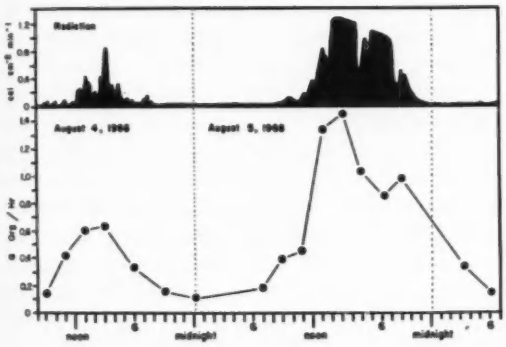

FIG. 13. Diurnal algal export on August 4 and 5, 1966, together with radiation measurements during this period.

contrasted with 0.91 organic matter per hour on August 5. This basic pattern of export during the summer months, and especially on clear days, was repeated throughout the summer and is largely responsible for the observed maximum during this period.

After numerous experiments shading portions of algal mats with black polyethylene, it was noted that upon sudden exposure to full sunlight, small bubbles, presumably oxygen, would appear within layers of algal filaments within minutes after the cover was removed. Similar observations were made on bright clear days when sunlight first reached the algal mats. These trapped bubbles raise a thin layer of mat up off the substrate surface or separate layers of accumulated mat, and when they escape, usually within minutes, they split and fragment a thin layer of mat which when in contact with the current tears off, resulting in increased export. Net collections affirm this hypothesis as samples are composed of small, torn fragments of algal material. This process of bubble formation is restricted to a short period when full sunlight first reaches the mat, and should not be interpreted as an event occurring throughout the day.

McIntire and Phinney (1965) measured export of biomass from laboratory streams, and noted peak periods to be associated with turbid water conditions. However, the greatest rate of export was recorded in early September, which they attributed to peak increases in biomass occurring in July and

August. Turbid water conditions were never encountered at the spring and the algal mats were dissimilar to the periphytic, epilithic (Aufwuchs) community studied by them. Teal (1957) and Odum (1957) discussed the importance of export in relation to the community energy budgets but did not give values for the seasonal pattern of export that can be compared with these values.

Grazing

Since data on the population dynamics of the dominant herbivore, *Hedriodiscus truquii*, are lacking, an accurate picture of grazing losses cannot be obtained from spring 3. However, from extensive studies of the population dynamics and energy flow of *Hedriodiscus* in adjacent springs, a reasonable approximation can be made (Stockner, MS, 1967).

Maximum and minimum densities of *Hedriodiscus* observed on accumulated mat in 1965 were of the order of 16/m² and 4/m², respectively. An average ingestion rate for *Hedriodiscus* (actually, it varied depending on instar stage and condition of larvae) determined from feeding experiments, was 15 mg algae/day (Stockner, MS, 1967). An approximation of grazing pressure then would most likely lie within the range of 240–60 mg algae/m² per day during the summer. It should be noted that values are expressed in milligrams wet weight, including ash. When expressed in units directly comparable with algal biomass data (Table VI), the values reduce to 15–3 mg organic matter/m² per day. In comparison with export losses these values seem insignificant, but nevertheless represent a loss of algae that must be considered when an energy budget is computed.

Energy Budget, Spring 3, 1966

The mean annual income and expenditure of energy was computed from estimates obtained in 1966 (Table VII). To convert these estimates of primary production expressed as grams O_2 to Kcal the values were multiplied by 3.4, the calculated caloric equivalent of 1 g of oxygen (Maciolek, 1962).

Table VII. The energy budget for spring 3, 1966. Values in Kcal/m² per year.

Income		
Primary production	4607	
Expenditure		*% total*
Community respiration	1158	25.1
Export	1461	31.7
Grazing	20	0.4
Deposition[a]	1968	42.8
Total	4607	

[a]Obtained by difference.

Of the yearly income of energy, loses attributable to community respiration and export (56.8%) represent the major losses, whereas grazing (0.4%) is hardly significant. By difference, an estimate of the amount of organic matter lost in travertine deposition (42.8%) can be obtained. This amount of energy (or organic matter equivalent) is not unrealistic nor is it surprising since it is obvious that the springs are continually growing and have been for thousands of years. Banks of travertine attest to the validity of this assertion.

The role of algae in travertine deposition has been known for some time, and mechanism(s) responsible have been suggested in this communication. It is clear from observation alone that large amounts of organic matter are continually lost during the gradual process of travertine formation and deposition. Thus, this estimate, though high, does not seem unrealistic in the light of present information.

In spring 3, normal travertine deposition was altered by continual harvests from the artificial substrate, but it is apparent from supplemental observation and experimentation on adjacent undisturbed springs throughout the 4-year study period, that deposition is an important figure in the yearly energy budget and must, therefore, be included in the derived balance of spring 3.

ACKNOWLEDGMENTS

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REFERENCES

AMERICAN PUBLIC HEALTH ASSOCIATION. 1965. Standard methods for the examination of water and waste water. 12th ed. APHA, New York. 769 p.

BEYERS, R. H., AND H. T. ODUM. 1959. The use of carbon dioxide to construct pH curves for the measurements of productivity. Limnol. Oceanog., **4**: 499–502.

BROCK, T. D. 1967. Relationship between standing crop and primary productivity along a hot spring thermal gradient. Ecology, **48**: 566–571.

BROCK, T. D., AND M. L. BROCK. 1967. The measurement of chlorophyll, primary productivity, photophosphorylation, and macromolecules in benthic algal mats. Limnol. Oceanog., **12**: 600–605.

CARPENTER, J. H. 1965. The accuracy of the Winkler method for dissolved oxygen analysis. Ibid., **10**: 135–143.

CARRITT, D. E., AND J. W. KANWISHER. 1959. An electrode system for measuring dissolved oxygen. Anal. Chem., **31**: 5–9.

CASTENHOLZ, R. W. 1967. Environmental requirements of thermophilic blue-green algae. Proc. Symp.: Environmental requirements of blue-green algae. U.S. Dept. Interior Publ. 111 p.

DUGDALE, V. A. 1967. Aspects of nitrogen nutrition of some naturally occurring populations of blue-green algae. Proc. Symp.: Environmental requirements of blue-green algae. U.S. Dept. Interior Publ. 111 p.

GORHAM, P. R. 1964. Toxic algae. *In* D. F. Jackson [ed.] Algae and man. Plenum Press, New York.

HANSEN, A. L., AND R. J. ROBINSON, 1953. The determination of organic phosphorus in sea water with perchloric acid oxidation. J. Marine Res., **12**: 31–42.

LEFEBVRE, M. 1964. Extracellular products of algae. *In* D. F. Jackson [ed.] Algae and man. Plenum Press, New York.

LENN, R. C. MS, 1966. Primary productivity of a thermal spring. M.Sc. Thesis. Univ. of Calif., Davis, Calif.

MACIOLEK, J. A. 1962. Limnological organic analyses by quantitative dichromate oxidation. U.S. Dept. Interior, Bureau Sports Fish. Publ., Res. Rept. No. 60. 61 p.

MCINTIRE, C. D., R. L. GARRISON, H. K. PHINNEY, AND C. E. WARREN. 1964. Primary production in laboratory streams. Limnol. Oceanog., **9**: 92–102.

MCINTIRE, C. D., AND H. K. PHINNEY. 1965. Laboratory studies of periphyton production and community metabolism in lotic environments. Ecol. Monographs, **35**: 237–258.

ODUM, H. T. 1956. Primary production in flowing waters. Limnol. Oceanog., **1**: 102–117.
1957. Trophic structure and productivity of Silver Springs, Florida. Ecol. Monographs, **27**: 55–112.

PAINE, R. T. 1966. Endothermy in bomb calorimetry. Limnol. Oceanog., **11**: 126–129.

ROBINSON, R. J., AND T. G. THOMPSON. 1948. The determination of nitrite in sea water. J. Marine Res., **7**: 42–48.

STEEMANN-NIELSEN, E. 1952. The use of radio-active carbon (C^{14}) for measuring organic production in the sea. J. Conseil, Conseil Perm. Intern. Exploration Mer, **18**: 117–140.

STOCKNER, J. G. MS, 1967. The ecology of the Ohanapecosh Hot Springs, Mount Rainier National Park, Washington. Ph.D. Thesis. University of Washington, Seattle, Wash.

TEAL, J. M. 1957. Community metabolism in a temperate cold spring. Ecol. Monographs, **27**: 283–302.

WESTLAKE, D. F. 1965. Comparisons of plant productivity. Biol. Rev., **30**: 385–425.

WHITFORD, L. A. 1960. The current effect and growth of freshwater algae. Trans. Am. Microscop. Soc., **79**: 302–309.

WINBERG, G. G. 1961. Production primaire du plankton lacustre, étudiée à l'aide de trois méthodes: d'oxygène, du carbonne radioactif et de la chlorophylle. Verh. Intern. Ver. Limnol., **14**: 109–112.

ZWICKER, B. M. G., AND R. J. ROBINSON. 1944. The photometric determination of nitrate in sea water with a strychnidine reagent. J. Marine Res., **5**: 214–234.

Relation between Protein Extractability and Free Fatty Acid Production in Cod Muscle Aged in Ice[1]

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ABSTRACT

Protein extractability decreased as free fatty acid (FFA) was produced in cod muscle aged in ice. The decrease was small compared with that occurring in frozen-stored muscle of similar FFA content. Prolonged extraction in neutral salt solution in the presence and absence of bovine serum albumin (BSA) showed that loss in protein extractability in muscle aged in ice was reversible through dissociation of inextractable material and that the presence of BSA, a FFA acceptor, favored greater dissociation. Ultracentrifugal patterns of protein extracted from ageing muscle showed increasing polydispersity. Phase contrast microscopy showed that the inextractable material contained muscle fragments consisting of bundles of myofibrils, some of full fiber width. These results indicate that in ageing muscle, interaction of contractile protein with FFA results in the formation of a cross-linking network within the muscle fiber causing resistance to fragmentation and to protein extractability, and that the observed smaller loss during ageing in ice is in part due to dissociation occurring during extraction. They suggest that in muscle aged in the frozen state, the reaction between contractile protein and FFA increases and the complexes formed are stabilized.

INTRODUCTION

We have previously postulated that protein–free fatty acid (FFA) interaction is involved in the loss in protein extractability that occurs in frozen-stored cod muscle (Anderson et al., 1965). Our hypothesis is an outgrowth of Dyer's original theory (Dyer and Fraser, 1959) that there is a relation between lipid hydrolysis and the loss in protein extractability that accompanies development of toughness in frozen-stored fish muscle. The hypothesis appears to be incompatible with the literature in that (1) the FFA content of cod muscle after prolonged storage in ice is about 50% of the total lipids (Lovern and Olley, 1962), a level at which, in frozen-stored muscle, protein extractability is approaching a minimum (Dyer and Fraser, 1959), whereas (2) loss in protein extractability during storage in ice is relatively small (Love et al., 1965).

The question arises, why is it that in the presence of similar FFA contents, loss of protein extractability is greater in frozen storage than in storage in ice? There are several possible explanations for this phenomenon: (1) FFA may be prevented from reacting with contractile protein in the intact muscle because the reactants are kept apart and react only during the homogenization and extraction process, or because the plasma proteins (so-called sarcoplasmic

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proteins) act as FFA acceptors, similar to serum albumins, and bind FFA preferentially; (2) FFA may react with contractile protein resulting in increased association of the proteins within the muscle fibers, but since the reaction involves electrostatic forces (Anderson et al., 1965), dissociation may occur when the homogenized muscle is exposed to an extractant of sufficient ionic strength to bring about dissolution of the intimately associated contractile proteins of fresh muscle; (3) interaction of contractile protein with FFA resulting in loss in extractability may take place to some extent, but conditions may be more favorable for the reaction in the frozen state; or (4) as Love has suggested (Love et al., 1965) loss in protein extractability in muscle aged in ice may not be related to FFA production.

This paper describes our efforts to test the first two possibilities by determining in muscle aged in ice the relation of the FFA content of the muscle to protein extractability, using our usual criterion of protein extractability; the extent to which FFA released during aging are associated with the sarcoplasmic (water soluble) proteins; and the effect on protein extractability of added bovine serum albumin (BSA) and of prolonged exposure to our usual extractant as well as to extractant of higher ionic strength.

EXPERIMENTAL

Gutted cod (*Gadus morhua*) weighing 15–25 lb each were obtained commercially. They had been stored in ice for not more than 1 day after capture. At the laboratory, they were decapitated, and any bits of viscera remaining in the abdominal cavities were removed. The fish were then packed in ice and stored in a 4 C room. They were kept well iced, but no attempt was made to replenish the ice in the abdominal cavities.

For each interval of storage in ice, six fish were filleted. Myotomes obtained from them by a technique previously described (Anderson et al., 1965) were pooled and mixed to obtain representative tissue samples for protein and lipid determinations. Muscle tissue samples were prepared and extracted in a cold laboratory at 1 C.

For determination of soluble protein content, muscle-extractant homogenates were prepared using a technique previously described (Anderson et al., 1963). Extractants for water soluble or sarcoplasmic protein (0.05 ionic phosphate buffer, *p*H 7.5) and the 0.5 ionic buffered KCl for total extractable protein have been previously described (Connell, 1958). The 0.8 ionic extractant contained 0.0157 M Na_2HPO_4, 0.0031 M KH_2PO_4, and 0.75 M KCl. For low ionic strength extracts, the tissue to extractant ratio was 10:90; for 0.5 and 0.8 ionic extracts the ratio was 10:390; and for double strength extracts 20:380. When extraction was carried out in the presence of BSA, fraction V powder obtained from Nutritional Biochemicals Corporation was added to the extractant before use and assayed in the extractant. In calculating its contribution to the total soluble protein content of the extracts, a correction was made for its dilution in the final volume of muscle-extractant homogenate.

For determination of protein readily extractable, a portion of muscle-extractant homogenate was centrifuged at 1100 *g* for 20 min in a refrigerated centrifuge at 1 C immediately after preparation. The remainder of the homogenate was stored overnight (20 hr) in a cold room (1 C) for determination of protein extractable after prolonged exposure to neutral salt solution (extractant). The stored homogenate was stirred magnetically for 5 min, and a portion was centrifuged as above. The protein content of supernatant fluids of centrifuged extracts was assayed by either a biuret method (Layne, 1957) or an automated method (Failing et al., 1960). Results were expressed as percent protein extractable, the total protein content of the muscle having been assayed on portions of muscle-extractant homogenates at all storage intervals. This was necessary not only because of biological variation, but because of changes in protein content due to water uptake from the ice; leaching of nitrogenous substances, organic solutes, salts, and water, a process which is aggravated by pressure of ice and other fish; autolysis, including proteolysis; and bacterial action.

The nonprotein nitrogen content of muscle samples at each storage interval was determined on the trichloracetic acid filtrates of low ionic strength extracts, using an automated Kjeldahl procedure (Ferrari, 1960).

To monitor changes in composition of readily extractable protein extracts, sedimentation patterns were obtained with a Spinco Model E analytical ultracentrifuge using schlieren optics.[2] Ultracentrifugation was carried out at a constant temperature of 1 C and a rotor speed of 50,740 rpm. Single sector cells with an optical light path of 12 mm were used.

Residues of centrifuged muscle-extractant homogenates were examined microscopically to follow possible changes in structure of inextractable material. Residues were suspended in a small amount of supernatant fluid after centrifugation. Wet preparations of the suspended residues protected by coverslips were examined by phase contrast microscopy. Photomicrographs of representative samples of residues were taken using a green interference filter.

Lipid extracts of myotomes were prepared by the method of Folch et al. (1957). Lipid extracts of the supernatant material of low ionic strength homogenates were prepared using the technique of Bligh and Dyer (1959). The protein extract was treated as water in this procedure. All lipid extracts were prepared in triplicate.

Phospholipid-free fractions of total lipid extracts were prepared by the method of Antonis (1965). Free fatty acid contents of phospholipid-free extracts were estimated using a slight modification of the colorimetric technique of Novák (1965), in which heptane was added to the chloroform extracts of FFA to give the chloroform–heptane ratio called for in the procedure.

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[2]The use of trade names is merely to facilitate descriptions; no endorsement is implied.

RESULTS AND DISCUSSION

Extraction Studies

FFA IN MUSCLE

There was a slow increase in FFA content of the muscle until about the 12th day of storage (Fig. 1). After this, there was rapid FFA formation similar

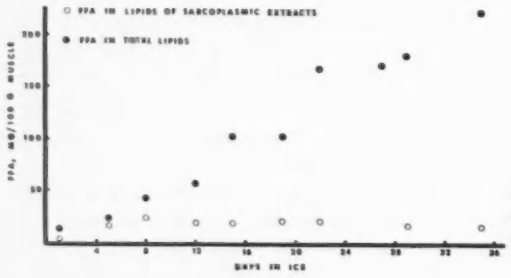

FIG. 1. Changes in FFA content of total lipids and lipids of sarcoplasmic protein extracts of cod muscle during storage in ice.

to that found by Lovern and Olley (1962). Values at given storage intervals were somewhat lower, however, than those for the statistical curve of the British investigators as well as those of a previous study at this laboratory where the fish were stored in melting ice in a room kept at a slightly higher temperature (6 C).

FFA ASSOCIATED WITH SARCOPLASMIC PROTEIN

The level of FFA associated with the sarcoplasmic fraction of muscle was lower than in whole muscle throughout storage (Fig. 1). If we correct for the variability in protein content of these extracts (Table I), it reached

TABLE I. Levels of FFA associated with sarcoplasmic protein extracted from cod muscle aged in ice.

Days in ice:	1	5	8	12	15	19	22	29	35
Protein N (g/100 g muscle extracted)	0.526	0.612	0.580	0.435	0.478	0.466	0.457	0.430	0.400
FFA (mg/g protein N)	7.6	20.6	42.1	45.5	39.7	44.8	44.4	39.8	39.8

a maximum after 12 days storage, at which time it plateaued until the last part of the storage period. Then, levels were slightly lower, probably due to oxidative changes. Determination of the FFA content of extracts of sarcoplasmic protein of muscle is subject to rather large errors due to the large dilution factor and the number of manipulations involved. The values used here indicate only the trend in this variable. Extraction of lipid from the

residue of low ionic strength homogenates was not attempted, since it is difficult to avoid aeration of the fragmented muscle even when a baffle technique is used. Evidence for oxidative changes with resulting lower yields in cell fractionation studies has been reported (Sheltawy and Olley, 1966).

Failure of sarcoplasmic protein to bind additional FFA after the 12th storage day is indirect evidence that further FFA binding was to contractile protein.

PROTEIN EXTRACTABILITY

Extractable protein content of muscle stored 5–12 days (Fig. 2) was in good agreement with the figure of Dyer et al. (1950) for maximum extractability (95%) in fresh cod muscle. The somewhat lower extractability in

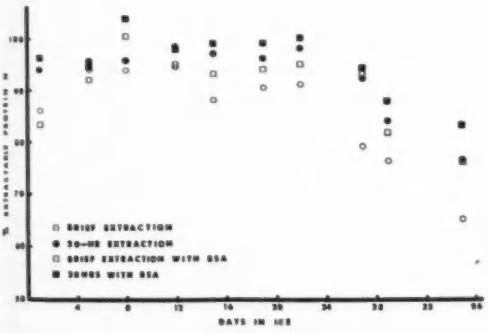

FIG. 2. Changes in extractable protein content of extracts of cod muscle stored in ice. Extracts were prepared in 0.5 ionic extractant with and without added BSA (0.9 g/10 g muscle extracted).

muscle stored 1 day was probably due to a requirement for an extractant of higher ionic strength (5% NaCl was used by Dyer) for maximum extractability in fresh postrigor muscle in which little softening has taken place. We have found an indication for this in a previous study (Anderson et al., 1965).

With brief extraction, extractable protein fell somewhat below the 95% level after the 12th day, the time of onset of rapid FFA production. It was not until after the 22nd day of storage, when FFA content of the muscle was high, that there was an appreciable decrease in protein extractability. Thereafter, the decrease was progressive and was related to increase in FFA content of the muscle. Extractability was much greater, however, than in muscle in which similar levels of FFA have been produced from lipid hydrolysis occurring during frozen storage (Dyer and Fraser, 1959).

Until the last third of the storage period, bacterial growth appeared to be light except at cut surfaces (visceral cavity and where the head had been severed). Even at the end of the storage period, gross observations suggested that changes deep within the muscle of these large fish were mainly autolytic. No build-up of nonprotein nitrogen was demonstrated.

Effect of bovine serum albumin.— With the usual short-extraction time technique for assessing protein extractability, the presence of BSA did affect

the amount of protein extracted (Fig. 2). Considerably more protein (6–17%) was extracted from muscle stored 27, 29, and 35 days when BSA was present in the extractant. But more protein was extracted from muscle aged 1 and 5 days when BSA was not present. In muscle of low FFA content, this is probably similar to what happens when you increase the muscle-extractant ratio. Less protein is extracted. This is particularly true with the use of 0.5 ionic extractant, when there is not quite enough salt to bring about immediate dissolution of fresh postrigor muscle.

Results obtained with short-extraction time (Fig. 2) could be taken as evidence that decrease in protein extractability is in part an artifact of the extraction technique. Homogenization in the extractant would cause extensive disorganization of the microstructure of the tissue and make most of the reactants available to each other. Decrease in soluble protein content when extraction is carried out in the absence of BSA would be related to insolubilization of contractile protein by interaction with FFA during the extraction procedure. The amount of protein insolubilized under these conditions in the presence of a given amount of FFA would be small in comparison with that occurring in muscle aged in the frozen state. This prediction is based on our studies in which we added C_{16} unsaturated fatty acids to muscle-extractant homogenates (Anderson et al., 1963). Greater yield of extractable protein in the presence of BSA would be due to prevention of interaction between contractile protein and FFA by BSA, a FFA acceptor. We have shown that in muscle-extractant homogenates containing added BSA, added linolenic acid was preferentially bound to BSA (Anderson et al., 1965). Decrease in amount of protein extracted in the presence of BSA from muscle aged 29 and 35 days would still need to be explained. It could not be attributed to protein–FFA acid interaction occurring during extraction, since sufficient BSA was present to bind all the FFA in the sample.

Effect of prolonged extraction.— Exposure of the homogenized muscle to the extractant, neutral salt solution, for 20 hr resulted in maximum protein extractability in samples stored 1–22 days, and in samples stored 27–35 days the percentages of extractable protein were considerably higher than those obtained with short extraction time (Fig. 2). With prolonged extraction time, there was almost always more protein extracted in the presence of BSA, with somewhat greater differences in the 29- and 35-day samples.

Data obtained with prolonged extraction time (Fig. 2) show that loss in protein extractability in muscle aged in ice is not related to insolubilization occurring during extraction. With prolonged extraction time values close to maximum extractability were obtained from muscle stored 1–27 days. This was true whether BSA was present or not. Moreover, the amount of protein extracted on short exposure to extractant containing BSA approached that obtained after prolonged exposure to extractant containing no BSA. Thus, the usual short-time exposure of the homogenized muscle to extractant with BSA had the same kind of effect as longer exposure to the extractant alone. With 29- and 34-day samples, this was especially noticeable.

Effect of extractant of higher ionic strength.— Table II shows differences in protein extractability from muscle homogenized in 0.5 and 0.8 ionic extractants after prolonged storage in ice. Generally, greater yields of soluble protein were obtained in 0.8 ionic extractant at all stages of the extraction process studied.

TABLE II. Effects of ionic strength of extractant on percentage protein extractable.

	Time in 0.5 ionic extractant (*hr*)			Time in 0.8 ionic extractant (*hr*)		
Days in ice:	0	20	20 R[a]	0	20	20 R[a]
25	69.9	82.8	97.3	87.0	95.7	102.4
33	53.9	63.3	90.1	76.9	82.1	94.8
39	50.8	60.5	81.1	61.7	69.2	81.0

[a]Samples reblended (two 5-sec blending periods separated by a 5-sec interval to allow for settling) after 20 hr extraction.

Taken together, the data on protein solubility show that loss in protein extractability in muscle aged in ice is a reversible process. The conditions under which it is reversed, prolonged exposure to the extractant, neutral salt solution with and without BSA, point to reversibility through dissociation. The cooperative effect of BSA, a free fatty acid acceptor, implicates protein–FFA complex formation in the process that results in loss of extractability. Increased extractability in neutral salt solution of higher ionic strength is additional evidence for dissociable complex formation as the reason for loss of extractability in muscle aged in ice. This interpretation is well supported by the literature. There is ample evidence for the combination of proteins with detergents and FFA anions, for the electrostatic nature of the combination, for diminished binding of such anions in the presence of increasing concentrations of neutral salt, and for the tendency of the complex to dissociate in medium of high ionic strength (Putnam, 1948; Bull and Reese, 1967).

Table II shows for muscle aged 25 and 33 days in ice greater increase in protein extractability resulting from increasing the ionic strength of the extractant than from prolonging the extraction time. These results suggest that dissociation is breaking down resistance to fragmentation during homogenization. Further evidence for a breaking down of resistance to fragmentation by exposure to salt solution was obtained when muscle exposed to the extractant for 20 hr was reblended. Table II shows that brief reblending resulted in maximum protein extractability from muscle aged in ice for 25 days, whereas for 33- and 39-day muscle, there were large increases in extractable protein. Reblending after prolonged exposure to 0.8 ionic salt solution after homogenization resulted in maximum protein extractability from muscle aged 25 and 33 days. This is evidence that resistance to fragmentation is

broken down by prolonged exposure of the homogenized muscle to salt solution. In muscle aged 39 days there was evidence that the association process is more extensive since reblending does not result in maximum extractability. No attempts were made to bring about greater dissociation in this muscle.

It could be argued that this increased protein extractability would result from additional blending without prolonged exposure to salt solution. But the percentages of protein extractable after additional blending before and after 20-hr exposure to salt solution were:

Days in ice	Before	After
25	75.4	97.3
33	65.2	90.1

If we assume an additional 10% increase in extractable protein resulting from 20-hr extraction time for samples in column 2, additional blending is evidently more effective when carried out after prolonged exposure of the homogenized muscle to extractant. One additional factor should be mentioned. The insoluble residue of muscle-extractant homogenates swells somewhat during ageing. This increases the chances for encounter of insoluble particles with the blender blades. This effect should be small, however, during this brief time of reblending.

Ultracentrifugal Studies

There was a very gradual change in the pattern of extracts prepared with short extraction time through the 25th day of storage (Fig. 3A–F). Comparison of patterns of extracts of 1-day (A–C) and 25-day (D–F) muscle shows a small decrease in peak II, the actomyosin fraction in this system, an increase in the gel fraction (pellet material), and a build-up of rapidly sedimenting material.

The first evidence for a marked change in composition was obtained with an extract of muscle aged 33 days. Figure 3G–I shows a greater decrease in peak II and a shoulder in the fast-moving material. The pattern for an extract of muscle aged 39 days (J–L) shows little further change.

Observation of the schlieren patterns of 33-day extracts during centrifugation showing this rapid build-up of fast sedimenting material suggested an extremely polydisperse system with components of varying but very high sedimentation rates — rates which were increasing in the cell area just ahead of the boundary due to dilution effects and decreasing in the area close to the pellet due to concentration effects. The pattern for an extract of the same muscle (Fig. 4A–C) prepared by prolonged exposure to the extractant and followed by brief reblending — an extract of somewhat higher soluble protein content — shows an increase in size of peak II and a much larger gel fraction. Again there is evidence for a shoulder in the fast sedimenting material which appeared earlier in the pattern.

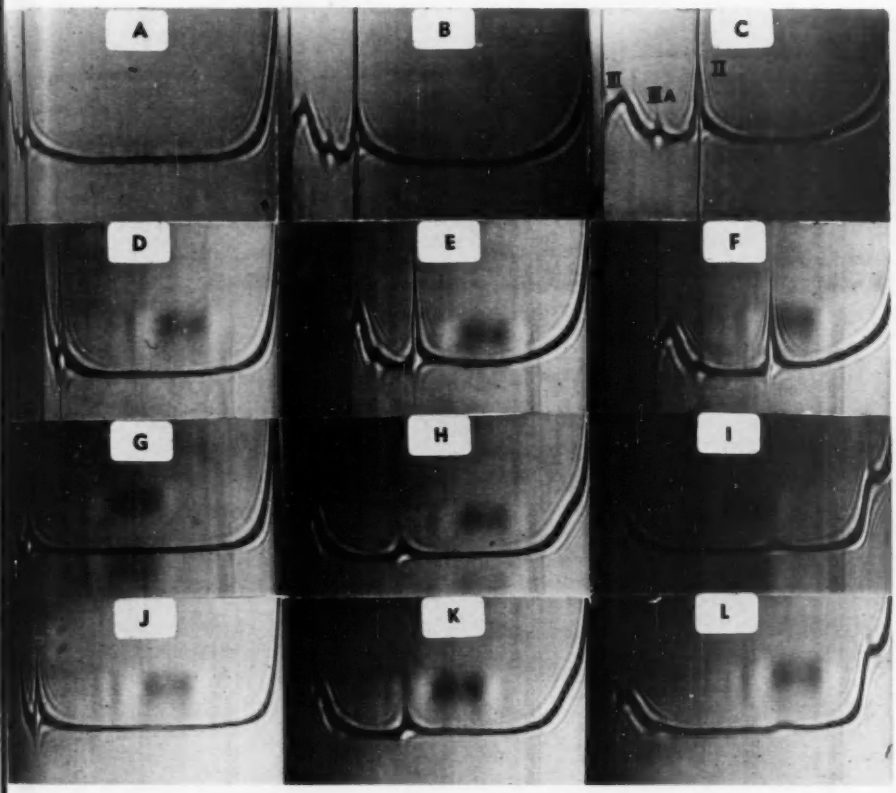

Fig. 3. Sedimentation patterns of protein extracts prepared with brief extraction time from muscle stored in ice for four periods. Extract of muscle aged in ice 1 day, protein concentration 0.556 mg protein N/ml: (A) 5 min; (B) 13 min; (C) 21 min. Extract of muscle aged 25 days, protein concentration 0.493 mg protein N/ml: (D) 0.5 min; (E) 8.5 min; (F) 16.5 min. Extract of muscle aged 33 days, protein concentration 0.388 mg protein N/ml: (G) 0.5 min; (H) 8.5 min; (I) 16.5 min. Extract of muscle aged 39 days, protein concentration 0.395 mg protein N/ml: (J) 0.5 min; (K) 8.5 min; (L) 16.5 min. The components are identified by Roman numerals in the order of decreasing sedimentation rates according to the system of King (1966). Photographs were taken at a phase plate angle of 55° after reaching a speed of 50,740 rpm.

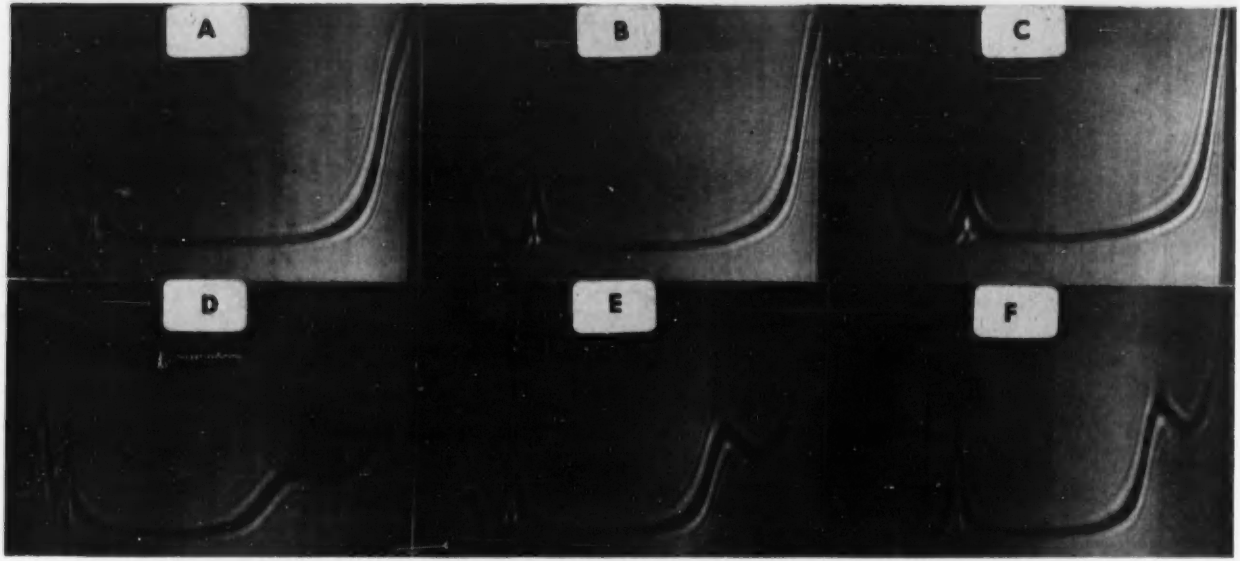

FIG. 4. Sedimentation patterns of protein extracts prepared with prolonged extraction time (20 hr) followed by brief reblending (two 5-sec blending periods separated by a 5-sec interval to allow for settling). Extract of muscle aged 33 days, protein concentration 0.673 mg protein N/ml (muscle to extractant ratio 10:390): (A) 0.5 min; (B) 4.5 min; (C) 8.5 min. Extract of muscle aged 39 days, protein concentration 1.21 mg protein N/ml (muscle to extractant ratio 20:380): (D) 8 min; (E) 16 min; (F) 24 min. Photographs were taken at a phase plate angle of 55° after reaching a speed of 50,740 rpm.

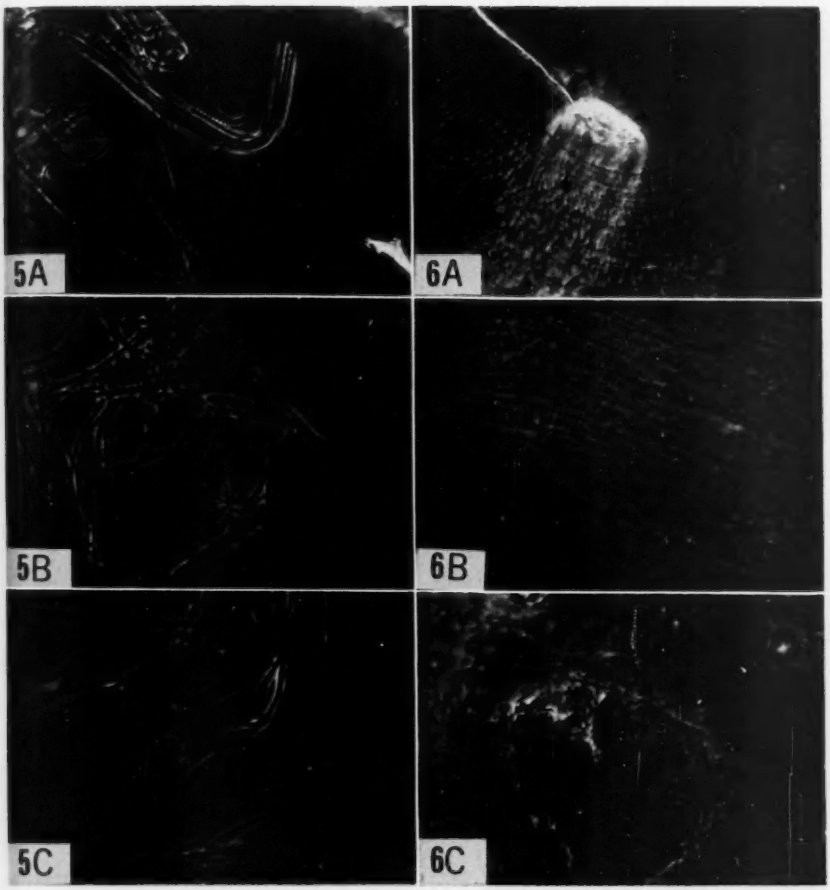

FIG. 5. Phase-contrast micrographs showing typical fragment size in residues of muscle homogenized in 0.05 ionic phosphate buffer after ageing in ice: (A) 25 days; (B) 33 days; (C) 39 days.
FIG. 6. Phase-contrast micrographs showing typical larger fragments in residues of muscle homogenized in extractant after ageing in ice 25 days: (A) after short extraction time in 0.5 ionic extractant, portion of fiber showing myofibrils protruding from severed sarcolemma; (B) after 20-hr exposure to 0.5 ionic extractant, portion of fiber showing partial dissolution of bands; (C) after 20-hr exposure to 0.8 ionic extractant, fragment of fiber.

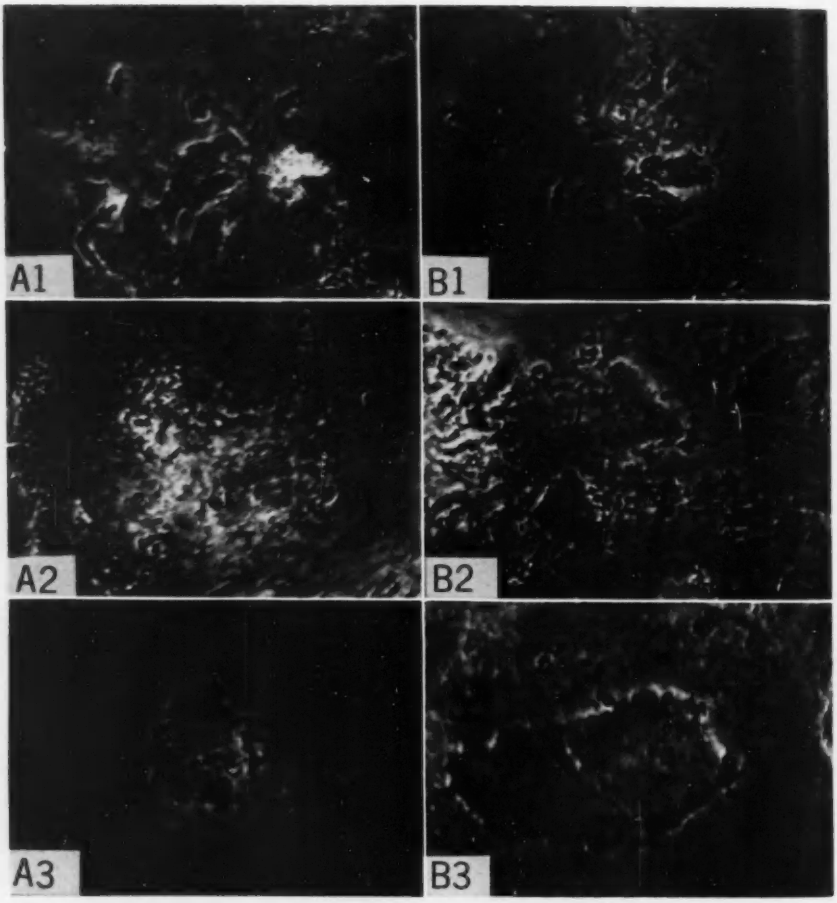

FIG. 7. Phase-contrast micrographs showing predominant size fragments in residues of muscle homogenized after ageing in ice. (A) 33 days, in 0.5 ionic extractant and (B) 39 days, in 0.8 ionic extractant: (1) after brief extraction time; (2) after 20-hr extraction time; (3) after reblending after 20-hr extraction time.

For 39-day muscle double strength extracts were prepared with 20-hr extraction time followed by brief reblending. Figure 4D–F shows that increase in concentration of all components resulted in a sharpening of boundaries and a resolving of a fast-moving peak in the area close to the pellet.

Thus, ultracentrifugal patterns of extracts of muscle precentrifuged at 1100 *g*, i.e. made up of proteinaceous material soluble by the criterion of solubility used in this study, showed the presence of increasing amounts of very rapidly sedimenting material (polydisperse material plus gel, intermediates between peak II and inextractable material sedimented at 1100 *g*) as aging of the muscle in ice progressed. It is unfortunate that patterns of double strength extracts were not obtained throughout the storage period.

A number of things make this an anomalous system — the very high rate of sedimentation and polydispersity of the intermediates that are of interest, difficulty in determining concentrations in the various areas of the cell, presence of a dissociating system, to name but a few. Since techniques for studying a system as anomalous as this have not yet been developed, the data must be treated as qualitative. They are still useful, however, in describing gross changes.

Microscopic Examination of Residues of Muscle-Extractant Homogenates

Examination of residues of low ionic strength muscle-extractant homogenates provided little evidence for resistance to fragmentation, even in muscle aged 25–39 days (Fig. 5).

Examination of residues of higher ionic strength homogenates (Fig. 6 and 7) proved more fruitful since presumably proteins making up the fragmented fibers from muscle not damaged during ageing had gone into solution, and only material that was inextractable showed up. Volume of residue, of course, increased with decreased protein concentration. Increasing numbers of fragments of full fiber width were found in samples from muscle aged 25–39 days after brief and prolonged extraction. Figure 6A shows the edge of a broken-off fiber with myofibrils spilling out beyond the severed sarcolemma. Figure 6B shows partial disappearance of structural bands at the broken-off end of a fiber and Fig. 6C a typical large fragment, consisting of several myofibrils in width and thickness. Few fragments of full fiber width were present in residues of reblended extracts, especially when 0.8 ionic extractant was used. However, the predominant fragment size was much smaller in all residues. Figure 7A and B shows typical microscopic fields observed in residues from muscle aged 33 days and 39 days respectively. The effects of extraction time and of reblending after prolonged extraction time on fragment size and appearance are discernible. With short extraction time banding was visible in most fragments, though there was more blurring of the banding pattern in 39-day muscle. With prolonged extraction, blurring increased so that the banding pattern was evident only in larger fragments. In reblended samples, where the volume of residue was relatively small, larger fragments with rather distinct banding were more frequently encountered.

Evidence obtained by microscopy substantiates that obtained by protein extractability studies. With increasing age of the muscle there was greater cohesion of myofibrils as evidenced by the increasing number and size of fragmented muscle fibers in the inextractable residue.

CONCLUSIONS

The data presented in this study support the conclusions that in cod muscle aged in ice: (*a*) contractile protein–FFA interaction takes place in situ; (*b*) the rate at which FFA's are formed and subsequently react with contractile protein increases after saturation of binding sites of sarcoplasmic protein for FFA; (*c*) contractile protein–FFA interaction results in formation of a cross-linked network within the muscle fiber that manifests itself in resistance of the muscle to fragmentation and to protein extractability; (*d*) the cross-linking process takes place through the formation of intermediates — protein complexes on their way to becoming inextractable — and is not a one-step transition from native to inextractable protein; (*e*) loss of protein extractability in the presence of large amounts of FFA is small because dissociation of cross-linkages is taking place in the extractant; (*f*) ionic bonding is the primary force in the formation of the protein–FFA complex since hydrophobic forces are largely unaffected by ionic strength.

The data suggest the possibility that greater loss in protein extractability in muscle aged in the frozen state is due to increased reaction rate between protein and FFA and stabilization of the complexes formed.

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REFERENCES

ANDERSON, M. L., F. J. KING, AND M. A. STEINBERG. 1963. Effect of linolenic, linoleic and oleic acids on measurement of protein extractability from cod skeletal muscle using the solubility test. J. Food Sci., **28**: 286–288.

ANDERSON, M. L., M. A. STEINBERG, AND F. J. KING. 1965. Some physical effects of freezing fish muscle and their relation to protein-fatty acid interaction. *In* R. Kreuzer [ed.] Technology of fish utilization. Fishing News (Books), Ltd., London. p. 105–110.

ANTONIS, A. 1965. Semiautomated method for the colorimetric determination of plasma free fatty acids. J. Lipid Res., **6**: 307–312.

BLIGH, E. G., AND W. J. DYER. 1959. A rapid method of total lipid extraction and purification. Canadian J. Biochem. Physiol., **37**: 911–917.

BULL, H. B., AND K. BREESE. 1967. Denaturation of proteins by fatty acids. Arch. Biochem. Biophys., **120**: 309–315.

CONNELL, J. J. 1958. Studies on the proteins of fish skeletal muscle, 5. Molecular weight and shape of cod fibrillar proteins. Biochem. J., **70**: 81–91.

DYER, W. J., AND D. I. FRASER. 1959. Proteins in fish muscle. 13. Lipid hydrolysis. J. Fish. Res. Bd. Canada, **16**: 43–52.

DYER, W. J., H. V. FRENCH, AND J. M. SNOW. 1950. Proteins in fish muscle. 1. Extraction of protein fractions in fresh fish. Ibid., **7**(10): 585–593.

FAILING, J. F., M. W. BUCKLEY, AND B. ZAK. 1960. Automatic determination of serum proteins. Am. J. Clin. Pathol., **33**: 83–88.

FERRARI, A. 1960. Nitrogen determination by a continuous digestion and analysis system. Ann. N.Y. Acad. Sci., **87**(2): 792–800.

FOLCH, J., M. LEES, AND G. H. SLOANE STANLEY. 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem., **226**: 497–509.

KING, F. J. 1966. Ultracentrifugal analysis of changes in the composition of myofibrillar protein extracts obtained from fish and frozen cod muscle. J. Food Sci., **31**: 649–663.

LAYNE, E. 1957. Spectrophotometric and turbidimetric methods for measuring proteins. *In* Sidney P. Colowick and Nathan O. Kaplan [ed.] Methods in enzymology. Vol. III. Academic Press, Inc., New York. 450 p.

LOVE, R. M., M. M. AREF, M. K. ELERIAN, J. I. M. IRONSIDE, E. M. MACKAY, AND M. G. VARELA. 1965. Protein denaturation in frozen fish. X. Changes in cod muscle in the unfrozen state, with some further observations on the principles underlying the cell fragility method. J. Sci. Food Agr., **16**: 259–267.

LOVERN, J. A., AND JUNE OLLEY. 1962. Inhibition and promotion of post-mortem lipid hydrolysis in the flesh of fish. J. Food Sci., **27**: 551–559.

NOVÁK, M. 1965. Colorimetric ultramicro method for determination of free fatty acids. J. Lipid Res., **6**: 431–433.

PUTNAM, F. W. 1948. The interaction of proteins and synthetic detergents. Advan. Protein Chem., **4**: 79–122.

SHELTAWY, A., AND J. OLLEY. 1966. Lipid distribution and recovery during salt fractionation of cod muscle. J. Sci. Food Agr., **17**: 94–100.

Use of EDTA Compounds for the Preservation of Haddock Fillets[1]

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ABSTRACT

Studies of the effect of treatment of haddock (*Melanogrammus aeglefinus*) with solutions of sodium salts of ethylenediaminetetraacetic acid (EDTA) have shown that a 1% solution of tetrasodium EDTA used as a dip can extend the storage life in ice (0 C) for 11 days over that of untreated controls. The texture of these fillets remained acceptable for the whole of their storage lives.

Fillets treated with 0.5 and 0.75% tetrasodium EDTA had a storage life extension of 5 days and those treated with 1% disodium EDTA had a storage life of 6 days longer than the untreated control fillets. Fillets treated with 0.5 and 1.0% disodium calcium EDTA solution showed 0- and 1-day storage life extension respectively.

Increase in trimethylamine (TMA) during storage was reduced by treatment with EDTA with the tetrasodium EDTA-treated fillets having the lowest rate of increase. Samples were rejected by the taste panel as spoiled with TMA values of between 3 and 9.5 mg TMA nitrogen (TMA–N) per 100 g.

Although treatment of fillets with EDTA extended the storage life in ice and suppressed the increase in TMA, there was little or no effect on the growth of the bacterial population.

INTRODUCTION

The most important cause of spoilage of fresh fish held in ice is growth of microorganisms. These spoilage organisms are picked up by the fillets, during processing, from the surface of the round fish by way of the cutting boards, knives, and hands of the filleter. The growth of these organisms during subsequent storage at ice temperature results in development of off-odors and off-flavors which are the indicators of spoiled fish. With careful handling to reduce contamination to a minimum, the fillets stored at ice temperature will remain edible for up to 15 days.

This storage period can be extended by applying a chemical preservative to slow the rate of growth of the microorganisms or to delay their use of the fish muscle as a nutrient. In the past, sodium nitrite, in amounts of up to 200 ppm of the fillets, has been used for this purpose. In 1959, the Canadian Food and Drug Act was amended and the use of sodium nitrite was no longer permitted as a preservative for fish.

In 1950, wide-spectrum antibiotics became available and the potential value of antibiotics of the tetracycline group for the preservation of fish was shown by Tarr et al. (1950).

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Recent work by Levin (1967) has shown low concentrations of tetrasodium ethylenediaminetetraacetic acid (Na_4EDTA) in nutrient broth to be effective in inhibiting the growth of three *Pseudomonas* strains of microorganisms involved in fish spoilage. He has also demonstrated the usefulness of a 1% EDTA dip for prolonging the storage life of haddock fillets.

Disodium EDTA (Na_2EDTA) and disodium calcium EDTA ($Na_2CaEDTA$) are recognized food additives for chelating metals in both United States and Canada. In Canada, the Food and Drug Directorate permits the use of Na_2EDTA in four foods and $Na_2CaEDTA$ in six food products. $Na_2CaEDTA$ is permitted in canned shrimp and tuna to a maximum level of 250 ppm and in canned crabmeat, lobster, and salmon to a level of 275 ppm to retard struvite formation and promote color retention. In the United States these additives are permitted in a larger number of foods, in amounts up to 500 ppm. However, neither country has provision in their regulations for the use of Na_4EDTA.

The present paper was designed to show the effect of Na_2EDTA, Na_4EDTA, and $Na_2CaEDTA$ dip on the organoleptic spoilage pattern of haddock fillets before and after cooking when stored in ice.

METHODS

Organoleptic Evaluation of Quality

After removal from storage the fillets were examined organoleptically and the odor, appearance, and texture recorded.

The fillets were then baked at 500 F (260 C) in separate pans covered with aluminum foil to prevent drying. The aluminum foil was not permitted to come in contact with the fish. Iced fillets were cooked for 12 min and the frozen control samples for 20 min.

The taste, texture, odor, and overall grade of stored samples were judged by a trained analytical taste panel as outlined by Dyer and Dyer (1949). A total of 24 judgments were made for each quality assessment and samples were assessed twice a week until spoiled. The scores assigned to samples were between 0 for a very badly spoiled sample and 100 for a perfect sample. Samples with a score of 40 or below were of unacceptable quality.

The mean values, standard error of the mean, and the 95% confidence limits were calculated (Snedecor, 1956) and curves were plotted using the mean values.

Trimethylamine Analysis (TMA)

The TMA values were determined by a method described by Dyer (1945, 1959).

Bacterial Plate Counts

Fillets were blended with twice their weight of distilled water in a sterile Waring Blendor for 1 min and then dilutions were made with distilled water

of this homogenate for bacterial counts. Plates were poured using the standard pour plate method using trypticase glucose extract agar medium.[2] Plates were incubated for 72 hr at 25 C before counting with a Quebec Colony Counter. Results were expressed as bacteria per gram of fish.

EDTA Determinations

EDTA determinations were made by a modification (Sinclair and Power, 1968) of a method proposed by Darby (1952) for colorimetric determination of sodium salts of EDTA in low concentrations.

EXPERIMENTAL

The fish used for this experiment were medium-size, good quality haddock (*Melanogrammus aeglefinus*) fillets obtained from a local fish processor. They were cut from fish 3–4 days old which had been caught on Georges Bank. Individual fillets weighed approximately 275 g.

The EDTA dips were prepared with distilled water in glass containers. The volume of each dip was 3 liters in which between 30 and 35 fillets were dipped. The fillets were allowed to remain in the solutions for 20 sec and after removal, allowed to drain for a further 20 sec. The fillets were then packaged individually in heat-sealed bags[3] without vacuum. Untreated fillets for controls, were also packaged in the same manner. All samples were stored in melting ice at 0 C. Samples of the same fish were also packaged in 1-lb boxes and frozen in a pre-chilled plate freezer (Dole Freze-Cel Model 2735-6) with a refrigerant temperature of −40 C for use as a reference by the taste panel. These frozen samples were stored at −26 C subsequent to freezing.

Samples were removed from storage and the quality assessed twice weekly for chemical, microbiological, and organoleptic examination of the raw and cooked fillet. The experiment was continued until the samples were no longer acceptable to the taste panel.

Solutions of the following concentrations of the various sodium salts of EDTA were assessed for their effectiveness as preservative dips for haddock fillets: (1) 0.5% Na_4EDTA; (2) 0.75% Na_4EDTA; (3) 1.0% Na_4EDTA; (4) 1.0% Na_2EDTA; (5) 0.5% $Na_2CaEDTA$; (6) 1.0% $Na_2CaEDTA$. The *p*H of the various solutions is shown in Table I.

RESULTS

Organoleptic Evaluation of Quality

ASSESSMENT OF RAW FILLETS

Untreated control fillets exhibited very similar spoilage patterns in all experiments.

After 4 days storage the odor was fresh to just slightly sweet, changing after 8 days to sweet. At 10 days, the untreated fillets had a strong sweet

[2]Baltimore Biological Laboratory, Baltimore, Maryland.
[3]Laminated, cellophane-polyethylene plastic.

TABLE I. *p*H of EDTA salts in solution.

Contents of dip	Concentration (%)	*p*H
Na_2EDTA	1.0	4.7
Na_4EDTA (Fisher Reagent)	1.0	10.6
Na_4EDTA (Na_3EDTA + NaOH)	1.0	11.4
Na_4EDTA (Na_3EDTA + NaOH)	0.50	11.0
Na_4EDTA (Na_3EDTA + NaOH)	0.75	10.5
$Na_2CaEDTA$	1.0	6.6
$Na_2CaEDTA$	0.50	6.6

odor which became fishy and stale at 16 days. At this point the untreated fish was rejected.

The color remained the normal white–cream color of fresh fish until the 10th day of storage when it showed very slight greyish discoloration. At 14 days the discoloration had increased giving the fillets a patchy appearance.

In texture, the fillets were normal and firm at 4 days, showing slight separation of the fillet along the center line after 8 days storage. At 10 days, the fillets were ragged and slightly soft, becoming soft at 14 days.

Fillets dipped in 1.0% Na_2EDTA solution maintained a normal fresh odor for 17 days. After 20 days storage, the odor was slightly sour. Color was normal until 13 days when it became slightly darkened. The texture of these fillets was firm to 9 days, then remained slightly soft until fillets were rejected at 20 days.

Samples treated with 1% Na_4EDTA solution showed superior storage characteristics on ice to those dipped in the Na_2EDTA solution. The former had a normal fresh odor until after 17 days storage, after which the odor became slightly sour and stale. The odor became progressively more fishy and stale until the fillet was rejected at 28 days.

There was a noticeable firmness of texture in these samples until after 28 days of storage when fillets became soft and ragged.

Haddock fillets dipped in the 0.75% Na_4EDTA solution did not keep as well as those dipped in 1.0% solution, as they were unacceptable in the raw state after 20 days of storage. The odor of these fillets was fresh until 4 days storage, after which it became progressively sweeter until the fillets were rejected. Color was normal for 13 days, gray–cream at 18 days, and pale beige at 20 days when the fillets were rejected. Texture was firm at 4 days, becoming softer as storage progressed. At 7 days there was slight myotome separation and at 13 and 18 days the fillets were soft and ragged in appearance. At 20 days the texture became coarse.

Fillets dipped in 0.5% Na_4EDTA solution followed an almost identical storage pattern, and were unacceptable after the same storage period, 20 days. Treatment with 0.5 and 1.0% $Na_2CaEDTA$ solution produced very similar characteristics to those of the untreated fish.

Odors progressed from sweet to fishy during the storage period. Color was normal for the first 10–11 days, becoming greyish towards the end of the storage period. Texture, which was firm during the first part of the storage period, became rubbery and coarse. Both Na_4EDTA- and $Na_2CaEDTA$-treated fillets were rejected after 14–15 days.

The physical and organoleptic characteristics of the uncooked fillets are shown in Tables II, III, and IV.

TABLE II. Physical and organoleptic characteristics of uncooked haddock fillets treated with $Na_2CaEDTA$ or untreated as controls. (Sl. = slightly.)

Treatment	Time (*days*)[a]	Odor	Color	Texture
Untreated	3, 4	Normal, sl. sweet	Normal	Firm
	7, 8	Sweet	Normal	Firm, sl. separated
	10	Strong sweet	Very sl. darkened	Ragged, sl. soft
	14	Fishy–stale	Patchy-darkened	Soft
0.5% $Na_2CaEDTA$	3	Sl. sweet	Normal	Firm, sl. separated
	7, 8	Faint sweet	"	Firm, sl. separated
	10	Sweet	"	Tail ragged
	14	Fishy	"	Coarse, rubbery
1.0% $Na_2CaEDTA$	3	Sweet	"	Firm, tail sl. ragged
	7, 8	Sweet–sour	"	Firm, sl. separated
	10, 11	Sweet	"	Rubbery, sl. separated
	14, 15	Sweet, sl. fishy	Greyish	Coarse, sl. ragged

[a]In each case, raw fillets were unacceptable on last day.

ASSESSMENT OF COOKED FILLETS (TASTE PANEL EVALUATION)

Texture. — Samples with 0.5 and 0.75% Na_4EDTA and 0.5 and 1.0% $Na_2CaEDTA$ treatments were poorer in texture than the untreated control. The latter spoiled before marked texture deterioration. The poorer texture scores of these EDTA-treated samples appeared to be due to toughness. The texture of the samples treated with 1.0% Na_4EDTA and 1.0% Na_2EDTA solutions remained acceptable throughout the storage lives of the samples, although those with 1.0% Na_4EDTA treatment showed a definite firmness of texture throughout the storage period.

Taste. — All samples of haddock treated with EDTA showed improvement in taste over the untreated control, although in the case of samples treated with $Na_2CaEDTA$, the improvement was very slight. Fillets treated with 0.5 and 0.75% Na_4EDTA were acceptable in taste for some 4 days longer. Treatment with 1% Na_2EDTA solution gave an acceptable taste for some 7 days longer than control fillets. Na_4EDTA treatment of 1% gave acceptable taste scores for 11 days longer than control fillets. Changes in taste for EDTA-treated samples followed much the same pattern as did the control. Throughout

TABLE III. Physical and organoleptic characteristics of uncooked haddock fillets treated with Na_4EDTA or untreated as controls. (Sl. = slightly.)

Treatment	Time (*days*)[a]	Odor	Color	Texture
Untreated	3, 4	Normal, sl. sweet	Normal	Firm
	7, 8	Sweet	Normal	Firm, sl. separated
	10	Strong sweet	Very sl. darkened	Ragged, sl. soft
	14	Fishy–stale	Patchy darkened	Soft
1.0% Na_4EDTA	9	Normal	Normal	Firm
	13	"	Normal	"
	17	"	Very sl. darkened	"
	20	Very sl. sour–stale	Sl. darkened	"
	24	Fishy–stale	Sl. darkened	"
	28	Fishy–stale	Darkened	Soft, sl. ragged
0.5% Na_4EDTA	4	Normal	Normal	Firm, sl. separated
	7	Sl. sweet	"	Sl. ragged at edges
	10	Sl. fishy	"	Sl. soft, separated
	13	Sweet	"	Sl. soft, separated
	18	Strong sweet	Grey–cream	Coarse, soft
	20	Fishy	Beige	Coarse
0.75% Na_4EDTA	4	Normal	Normal	Firm, sl. separated
	6, 7	Sl. sweet	"	Sl. myotome separated
	10	Sweet hay	"	Sl. soft, ragged
	13	Sweet	"	Soft
	18	Sweet	Grey–cream	Soft, ragged
	20	Pungent, sweet	Beige	Coarse, hard

[a]In each case, raw fillets were unacceptable on last day.

TABLE IV. Physical and organoleptic characteristics of uncooked haddock fillets treated with Na_2EDTA or untreated as controls. (Sl. = slightly.)

Treatment	Time (*days*)[a]	Odor	Color	Texture
Untreated	3, 4	Normal, sl. sweet	Normal	Firm
	7, 8	Sweet	Normal	Firm, sl. separated
	10	Strong sweet	Very sl. darkened	Ragged, sl. soft
	14	Fishy–stale	Patchy darkened	Soft
1.0% Na_2EDTA	9	Normal	Normal	Firm
	13	"	Normal	Sl. soft
	17	"	Very sl. darkened	"
	20	Sl. sour	Sl. darkened	"

[a]In each case, raw fillets were unacceptable on last day.

the experimental periods some taste panel members found flavors variously described as slightly soapy, sweet, metallic, or acid in all EDTA treatments. These flavors, however, were reported as bland and not unpleasant. In the very late stages of spoilage, acetic acid-type odors were noticeable during cooking of samples treated with 1.0% Na_2EDTA solution.

Overall grade. — The frozen controls remained well within the limits of acceptability throughout the experimental periods. Scores ranged from 65 to 85%. The untreated controls gave similar taste panel results in all three series. The overall quality of these samples deteriorated most rapidly and was unacceptable at 14 days. There were decided drops in quality at 9–11 days of storage. Overall grade scores for 1.0% Na_2EDTA samples maintained acceptable quality for 20 days. A significant drop in quality occurred at 17–20 days, from 59 to 37%. Fillets treated with 1.0% Na_4EDTA had the same initial quality as those treated with Na_2EDTA but maintained acceptable quality for 25 days. A noticeable drop in quality occurred in the period between 20 and 24 days. Na_4EDTA treatments of 0.5 and 0.75% dropped significantly in quality at 14 days. Both were unacceptable at 19 days. Both $Na_2CaEDTA$ treatments showed similar quality changes. Fillets dipped in 1.0% solution had slightly lower initial quality (68%) than those dipped in 0.5% solution (75%). In both cases, a significant quality drop occurred at 10 days. Fillets treated with 0.5% $Na_2CaEDTA$ solution became unacceptable at 14 days, and those treated with the 1% solution at 15 days. The spoilage pattern of these fillets closely resembled that of the untreated iced controls.

Taste panel results are recorded graphically in Fig. 1–3.

Bacterial Growth on Treated Fillets

Bacterial growth on the frozen reference samples remained low for the whole storage period between 8100 and 180,000 counts per gram. The untreated control samples showed a steady increase in bacterial growth and became unacceptable to the taste panel before the total count reached 5×10^6 bacteria per gram of fish. Treatment of the fillets with the various EDTA solutions did not prevent growth or decrease the rate of growth of microorganisms on the fillets and the total count increased until the fillets were rated as unacceptable by the panel. The final counts when the fillets became unacceptable, for the fillets dipped in 1% Na_4EDTA, 1% Na_2EDTA, and 1%Na_2 CaEDTA were 129×10^6, 169×10^6, and 124×10^6 organisms per gram, respectively. This agrees with the findings of Levin (1967). Total plate counts for fillets treated with EDTA solutions are shown in Table V.

Increase in TMA in EDTA-treated Fillets during Storage in Ice

The TMA values remained low in the frozen samples for the whole experiment. All frozen samples contained less than 0.6 mg TMA nitrogen (TMA–N) per 100 g fish. The untreated control samples were low in TMA until after 9–10 days storage when they began to increase rapidly to a value of approximately 10 mg TMA–N per 100 g at 14 days when they were rejected by the

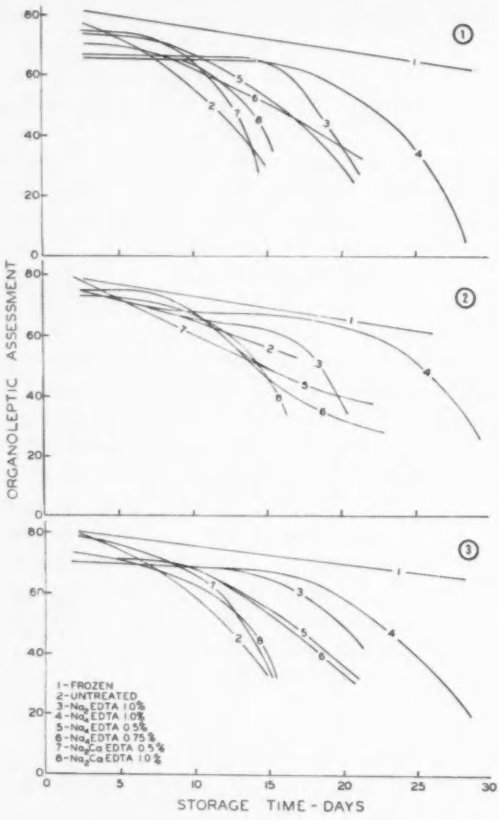

FIG. 1. Overall grade of EDTA-treated haddock fillets stored on ice (0 C).

FIG. 2. Texture changes in EDTA-treated haddock fillets stored on ice (0 C).

FIG. 3. Taste changes in EDTA-treated haddock fillets stored in ice (0 C).

taste panel. Increase in TMA in the samples treated with EDTA was less than in the untreated control. The fillets dipped in 1% Na_4EDTA showed the lowest rate of increase in TMA, remaining low for approximately 17 days before increasing slowly to a TMA content of 3 mg TMA–N per 100 g at 25 days when they were rejected by the taste panel. Samples dipped in 1% Na_2EDTA showed a slightly greater rate on increase in TMA than did the fillets dipped in 1% Na_4EDTA. These showed a rapid rate of TMA increase beginning at 17 days and reaching a value of 2.5 mg TMA–N per 100 g at 20 days when they reached the rejection point.

The increase in TMA in fillets treated with 0.5% Na_4EDTA, 0.75% Na_4EDTA, 0.5% $Na_2CaEDTA$, and 1% $Na_2CaEDTA$ was more rapid than in fillets treated with 1% Na_4EDTA and 1% Na_2EDTA but slower than the untreated controls. These samples showed increases in TMA starting at 10 days after catching and increasing to values of 9, 9.5, 2.5, and 2.5 mg TMA–N per 100 g fish respectively. These values were lower than those reported by

TABLE V. Total bacterial plate counts per gram of fish.

Storage time (*days after catching*)	Treatment			
	Frozen	Untreated	1% Na_2EDTA	1% Na_4EDTA
7	25,000	56,000	30,000	19,000
11	26,000	151,000	110,000	74,000
14	16,000	5×10^6	2.9×10^6	14,000
18	16,000	–	1.8×10^6	30×10^6
21	48,000	–	169×10^6	50×10^6
24	51,000	–	–	129×10^6
	Frozen	Untreated	0.5% Na_2EDTA	0.75% Na_4EDTA
3	39,000	144,000	79,000	140,000
6	37,000	400,000	65,000	120,000
10	23,000	1.3×10^6	421,000	340,000
13	180,000	1.3×10^6	3.3×10^6	1.50×10^6
17	26,000	–	13×10^6	10×10^6
20	52,000	–	127×10^6	98×10^6
	Frozen	Untreated	0.5% $Na_2CaEDTA$	1.0% $Na_2CaEDTA$
5	27,000	38,000	27,000	15,000
9	14,000	87,000	104,000	106,000
13	8,100	3.5×10^6	1.68×10^6	1.1×10^6
17	9,800	–	–	124×10^6

Dyer and Dyer (1949) who found their spoiling fillets had levels of 4–15 and spoiled fillets had levels of 15–30 mg TMA–N per 100 g of fillets. The EDTA-treated samples spoiled at relatively low TMA values but with a large bacterial population. Figure 4 shows the increase in TMA in fillets with storage.

RESIDUAL EDTA IN TREATED FILLETS

The fillets treated with 1% Na_4EDTA and with 1% $Na_2CaEDTA$ were analyzed for residual EDTA. Of the fillets dipped in 1% Na_4EDTA, one lot of six fillets had EDTA contents between 187 and 333 ppm, and a second lot of eight fillets had EDTA contents between 256 and 348 ppm.

Three lots of fillets dipped in 1.0% $Na_2CaEDTA$ solution had EDTA contents of between 240 and 370 ppm, 180 and 210 ppm, and 170 and 235 ppm respectively. The first lot of fillets were noticeably softer in texture than the other lots. These values are lower than those given by Levin (1967), possibly because of the firmness and size of the fillets. It was found that soft fillets had higher residual EDTA contents than firm fillets of the same size.

DISCUSSION

Correlation between taste panel grades and TMA values was good. Those samples which had the lowest rate of TMA increase also had the longest storage life although the TMA values of the EDTA-treated samples were between 3 and 9.5 mg TMA–N per 100 g when they were rejected by the panel. Regression curves (Fig. 5) show that for much of the storage period of these samples the TMA values remained low in spite of a rapid growth of the bacterial population.

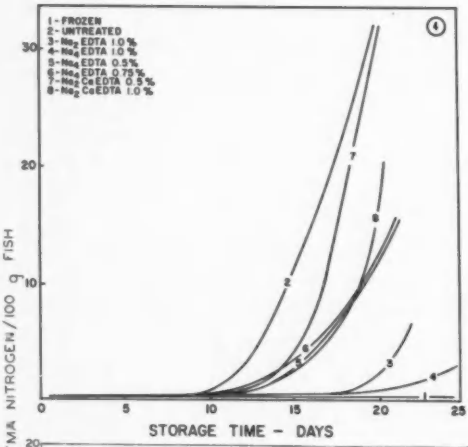
1 - FROZEN
2 - UNTREATED
3 - Na2 EDTA 1.0 %
4 - Na4 EDTA 1.0 %
5 - Na4 EDTA 0.5%
6 - Na4 EDTA 0.75 %
7 - Na2 CaEDTA 0.5 %
8 - Na2 CaEDTA 1.0 %
Mg TMA NITROGEN/100 g FISH
STORAGE TIME - DAYS

FIG. 4. Changes in TMA level in EDTA-treated haddock fillets stored in ice (0 C).

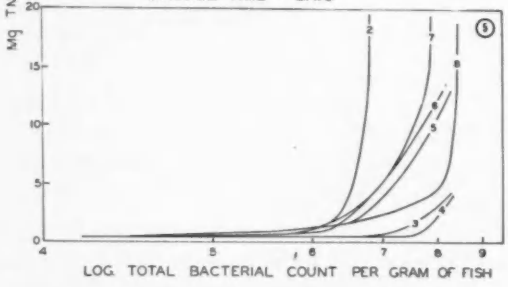

FIG. 5. Correlation between TMA level and total bacterial population for EDTA-treated haddock fillets stored in ice (0 C).

Treatment with 1.0% Na_4EDTA was the most successful in extending storage life of fillets (11 days extension), when taste panel scores were compared with those of untreated controls. The 1.0% Na_2EDTA gave 6 days extension, and the 0.75 and 0.5% Na_4EDTA treatments gave 5 days extension, whereas the $Na_2CaEDTA$ treatments gave no appreciable extension in storage life (Table VI).

The fact that uncharacteristic although not unpleasant tastes were noted in later stages of storage suggests that the normal spoilage pattern was altered by EDTA treatment. This idea is further reinforced by the fact that there

TABLE VI. Extension of storage life of haddock fillets as indicated by taste panel scores.

Treatment	Time of significant quality drop (*days*)	Storage life[a] (*days*)	Extension of storage life (as compared with untreated controls) (*days*)
Untreated	9–10	14	0
1% Na_2EDTA	17–20	20	6
1% Na_4EDTA	20–24	25	11
0.5% Na_4EDTA	14	19	5
0.75% Na_4EDTA	14	19	5
0.5% $Na_2CaEDTA$	10	14	0
1.0% $Na_2CaEDTA$	10	15	1

[a]Storage life is measured from date caught.

was a definite repression of TMA values in comparison with untreated controls, but at the same time there was no significant difference in total colony counts. It is possible that EDTA has a selective inhibitory action on the growth of TMA-producing bacteria, thus allowing competitive strains to grow at a faster rate.

Levin's work indicated that EDTA inhibited growth of three *Pseudomonas* strains in nutrient broth culture — *P. putrefaciens*, *P. fragi*, and *P. fluorescens* (Levin, 1967). *Pseudomonas putrefaciens* reduces TMA oxide to TMA. *Pseudomonas fragi* and *P. fluorescens* do not reduce TMA oxide although they are capable of causing other odor-producing changes in fish. It is also possible that EDTA inhibits certain bacterial enzyme activities, among these being the reduction of TMA oxide to TMA. In the presence of EDTA, alternate enzyme activities may be used and the rate of bacterial growth would not be affected. Castell (1949) found the latter hypothesis to be true in the case of nitrite-treated cod fillets.

The superior preservative action of the Na_4EDTA solution (*p*H 10.6) can probably be attributed to its greater chelating properties at high *p*H levels (Greigy Industrial Chemical, 1952).

Comparison of texture and taste score curves for the Na_2EDTA and Na_4EDTA treatments reveals that the latter had higher texture than taste scores. It appears that texture was significantly responsible for the higher overall grade maintained by the Na_4EDTA treatment as compared with the Na_2EDTA treatment.

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REFERENCES

CASTELL, C. H. 1949. Effect of nitrite on reduction of trimethylamine oxide in cod fillets. J. Fish. Res. Bd. Canada, **7**(7): 421–429.

DARBY, A. 1952. Colorimetric determination of the sodium salts of ethylene diaminetetra-acetic acid. Anal. Chem., **24**: 373–378.

DYER, W. J. 1945. Colorimetric determination of trimethylamine as the picrate salt. J. Fish. Res. Bd. Canada, **6**(5): 351–358.

1959. Report on trimethylamine on fish. J. Assoc. Offic. Agr. Chemists, **42**: 292–294.

DYER, F. E., AND W. J. DYER. 1949. Changes in the palatability of cod fillets. J. Fish. Res. Bd. Canada, **7**(8): 449–460.

GEIGY INDUSTRIAL CHEMICAL. 1952. Sequestrene. Geigy Industrial Chemicals, Ardsley, N.Y.

LEVIN, R. E. 1967. The effectiveness of EDTA as a fish preservative. J. Milk Food Technol., **30**(9): 277–283.

SINCLAIR, R. E., AND H. E. POWER. 1968. Colorimetric determination of EDTA salts in haddock, cod and flounder fillets. Fish. Res. Bd. Canada, Tech. Rept. No. 68.

SNEDECOR, G. W. 1956. Statistical methods. Iowa State University Press, Ames, Iowa.

TARR, H. L. A., B. A. SOUTHCOTT, AND H. M. BISSETT. 1950. Effect of several antibiotics and food preservatives in retarding bacterial spoilage of fish. Fish. Res. Bd. Canada, Pacific Progr. Rept. No. 83. p. 35–38.

Distribution of Fatty Acids in Cod Flesh Lipids[1]

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ABSTRACT

Cod flesh lipids were separated by silicic acid chromatography into eight fractions, and the fatty acid distribution in five of these was examined by gas–liquid chromatography (GLC). As compared with the fatty acid composition of total flesh lipids, sterol esters contained less 16:0 but more 20:5ω3; ethanolamine phosphatides contained less 16:0, less 20:5ω3, but more 22:6ω3; serine phosphatides contained less 16:0 and less 20:5ω3 but more 18:0; and choline phosphatides had a fatty acid composition roughly similar to that of total flesh lipid but containing slightly more 16:0 and 20:5ω3 and less 22:6ω3. In fatty acid composition, the triglycerides more closely resembled liver lipid than any of the flesh lipid fractions.

INTRODUCTION

The flesh of the Atlantic cod, *Gadus morhua*, contains 0.5–0.7% lipid. Analyses of lipid class distribution (Garcia et al., 1956; Bligh and Scott, 1966) have shown that this consists mainly of phosphatidyl choline (60% approx) with smaller amounts of other phospholipids and about 15–20% nonphospholipid. The major fatty acids present are 16:0,[2] 18:1ω9, 20:5ω3, and 22:6ω3, which together represent more than 80% of the total, with a wide range of other fatty acids present in relatively small amounts (Ackman and Burgher, 1964; Jangaard et al., 1967a).

Although the lipids are present only in small amounts in cod flesh, they have a considerable influence on the quality of stored fillets. Cold storage of cod, and of other "lean" fish, is accompanied by hydrolysis of the flesh lipids (Lovern et al., 1959; Olley and Duncan, 1965; Bligh and Scott, 1966). The resulting accumulation of free fatty acids causes deterioration of texture and quality of the fillets. The process has been shown to be autolytic rather than microbial in origin (Olley and Lovern, 1960). It has been shown in cod that the free fatty acids are liberated exclusively from phospholipid and that their composition is similar to that of the original lipid (Bligh and Scott, 1966; Olley and Duncan, 1965).

An essential preliminary step in a study of the course of autolysis is to establish the distribution of fatty acids among fresh cod flesh lipid fractions. Roubal (1967) has shown that important differences exist in the composition of some lipid fractions in the flesh of the Pacific cod (*G. macrocephalus*) but

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[2]Shorthand notation for chain length:number of double bonds and position of ultimate double bond relative to the terminal methyl group.

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similar information was not available for *G. morhua*. This paper describes the fatty acid composition of the major flesh lipids of *G. morhua*. The results are compared with those from similar analyses reported in the literature, and their relevance to the available data on lipid autolysis is discussed.

EXPERIMENTAL

The fish were caught by handline at Terence Bay, N.S., and were kept for some weeks in the laboratory live-holding facilities until October 1967. Four males and four females were selected and killed by a blow on the head. Weights and lengths were recorded and the fillets were removed and extracted immediately by the method of Bligh and Dyer (1959). No attempt was made to separate light and dark (lateral line) muscle. Livers were removed and extracted similarly. Iodine values (I.V.'s) of liver lipids were determined by Wijs' method.

Samples of flesh and liver lipid from each fish were transesterified with BF_3-methanol (Morrison and Smith, 1964). Samples of the methyl esters were examined by gas chromatography on columns of 15.5% EGSS-X on 100–120 mesh Gas Chrom P, and 15.5% EGSS-Y on 100–120 mesh Gas Chrom P, or 3% EGSP-Z on 100–120 mesh Gas Chrom P (all 6 ft $\times$ $\frac{1}{8}$ inch O.D. stainless steel). The gas chromatograph used was an Aerograph Hy-FI Model 500D fitted with a flame ionisation detector. Peaks were identified by comparison with standards, or by log-plot and separation factor procedures (Ackman and Burgher, 1965), and correction factors for quantitative determinations were applied (Ackman and Sipos, 1964). Finally, all samples were hydrogenated and re-run to confirm identification and quantitation of major peaks.

As analyses of total flesh lipid methyl esters showed that there was little variation from fish to fish within each sex, equal quantities of male or female lipids were pooled, and accurately weighed samples of about 300 mg lipid from each sex were subjected to column chromatography on silicic acid, broadly as described by Hirsch and Ahrens (1958), with a suitable reduction in scale. Rigorous precautions were taken to prevent oxidation during chromatography; the column was run under nitrogen pressure and the fraction collector was housed in a plastic bag inflated with nitrogen. The following fractions, chromatographically homogenous by thin-layer chromatography (TLC), were separated: sterol esters, triglycerides, sterols (mainly cholesterol as judged by TLC), polyglycerol phosphatide with some ethanolamine phosphatide, ethanolamine and serine phosphatides, (cephalins), and choline phosphatide. The final eluant used, methanol, eluted a small amount of what may have been sphingomyelin or lysophosphatidyl choline. No attempt was made to distinguish between diacyl phospholipids and plasmalogen forms. The course of elution was followed by TLC of eluted fractions, and fractions were identified by comparison with standards, and by reaction to specific spray reagents (ninhydrin and Dragendorff's reagent). The ethanolamine and serine phosphatides were separated by column chromatography on ammoniacal silica

gel (Spitzer and Balint, 1964). Recovery from both columns were within the range 95–105% of load.

Lipid fractions so obtained were weighed, transesterified, and the component fatty acids analysed as described above.

RESULTS AND DISCUSSION

The size of the fish used in the experiment, and their flesh and liver lipid contents are shown in Table I. (Liver analyses are shown here mainly for comparison with flesh triglyceride analyses, as discussed below). In the females, the gonads were undeveloped and difficult to distinguish from the corresponding male organs; this, and the small size of the fish concerned, suggested that they were immature. Local cod spawn in spring or autumn, and in either case, mature females would have shown some development of gonads.

TABLE I. Size of fish and lipid content of flesh and liver (mean ± standard error).

	Males	Females
Length (*cm*)	60±2.5	53±2.2
Weight (g)	1785±319.6	1086±233.4
Lipid in flesh (%)	0.59±0.054	0.62±0.041
Lipid in liver (%)	53.0±8.10	30.6±8.98

In the cod, depot fat is stored mainly in the liver, and the lipid content and fatty acid composition of this organ is variable, depending on the nutritional and reproductive status of the fish. In contrast, flesh lipid remains nearly constant in level and in composition (Jangaard et al., 1967a); this implies a metabolic function other than that of an energy reserve. Flesh and liver lipid levels of fish in the present experiment fell within the range of expected values. Liver lipid levels were variable, and it is of interest that in apparently immature fish, those of the females were lower than those of the males, as shown in October/November/December results of Jangaard et al. (1967b). Flesh lipid levels were similar to those reported by Jangaard et al. (1967a). The total fatty acid composition of flesh and liver lipids, shown in Table II, agreed closely with results published by Jangaard et al. (1967a) for October fish. The lower fat level in the female livers (Table I) was reflected proportionately in their lower levels of 20:1 and 22:1 (depot) fatty acids.

The distribution of lipid classes in cod flesh is shown in Table III. The composition agrees well with that reported by Bligh and Scott (1966). The absence of any free fatty acids is notable; this was attributed to the fact that fillets were extracted immediately after death, thus reducing the possibility of any lipid hydrolysis (cf. Lovern et al., 1959; Bligh and Scott, 1966).

TABLE II. Fatty acid composition of flesh and liver lipids (acids expressed as percentage of total C_{12}–C_{24}) (mean ± standard error).

Acid	Flesh		Liver	
	Male	Female	Male	Female
12:0	–	–	0.7±0.62	0.2±0.09
14:0	1.4±0.14	1.4±0.14	4.9±1.04	4.0±1.05
14:1 + Br 15:0[a]	Trace	Trace	0.3±0.05	0.5±0.30
15:0	0.3±0.04	0.3±0.05	0.4±0.06	0.5±0.09
15:1 + Br 16:0	0.1±0.03	Trace	0.1±0.09	0.2±0.09
16:0	19.6±0.37	20.4±0.15	12.4±0.67	12.7±0.53
16:1ω7[b]	3.5±0.09	3.6±0.18	11.6±1.50	14.1±1.17
16:2ω4	0.2±0.19	0.3±0.10	0.2±0.05	0.3±0.10
16:3 + Br 17:0	–	Trace	0.4±0.09	0.4±0.09
16:4	–	–	0.5±0.25	0.7±0.23
17:0	0.2±0.10	0.1±0.03	0.2±0.05	0.2±0.09
17:1 + Br 18:0	0.1±0.04	0.1±0.05	0.4±0.09	0.5±0.02
18:0	3.8±0.24	3.8±0.22	1.8±0.35	1.9±0.26
18:1ω9[b]	13.8±0.25	12.5±1.23	22.6±1.47	23.3±2.03
18:2ω6	0.7±0.07	0.6±0.09	1.4±0.34	1.1±0.07
18:3ω6	0.2±0.04	0.2±0.01	0.7±0.22	0.4±0.04
18:3ω3	0.1±0.04	0.1±0.01	1.3±0.60	0.4±0.04
18:4ω3	0.4±0.06	0.2±0.08	1.9±0.50	1.8±0.34
20:1ω9[b]	3.0±0.38	2.4±0.04	7.6±1.79	6.4±2.17
20:2ω6	Trace	0.1±0.40	Trace	Trace
20:4ω6	2.5±0.72	2.7±0.57	0.5±0.15	1.5±0.90
20:4ω3	0.6±0.12	0.6±0.23	0.5±0.28	1.1±0.04
20:5ω3	17.0±0.49	17.6±0.73	12.6±2.14	13.2±1.37
21:5ω2	0.1±0.06	Trace	0.2±0.24	0.7±0.38
22:1ω11[b]	1.0±0.13	1.4±0.64	5.2±1.73	3.4±1.10
22:5ω6	0.3±0.26	0.2±0.05	–	–
22:5ω3	1.3±0.29	1.7±0.24	1.0±0.45	1.1±0.27
22:6ω3	29.8±1.05	29.6±2.22	10.6±2.12	9.3±1.53
24:1	Trace	Trace	Trace	Trace

[a]Br = Branched chain.

[b]Other isomers may be present (cf. Ackman et al., 1967).

The distribution of fatty acids among cod flesh lipid fractions is shown in Table IV. It is clear that the composition of triglycerides differed considerably from that of the other fractions, particularly in the levels of monoenes (of which there are more) and of polyunsaturates (of which there are less). In fatty acid composition, the flesh triglycerides (calculated I.V. ~ 124) more closely resembled liver lipid (Wijs I.V. ~ 154), and indeed, the levels of 20:1ω9 and 22:1ω11 (higher in male than in female livers) were reflected proportionately in flesh triglycerides. These results suggest that the triglycerides of cod flesh are derived directly from liver lipid.

TABLE III. Lipid class distribution in flesh (as percentage of total flesh lipid).

	Male	Female
Sterol esters	2.2	2.8
Triglycerides	4.1	3.6
Cholesterol	6.3	6.5
Polyglycerol phosphatide with some ethanolamine phosphatide	3.4	2.4
Ethanolamine phosphatide	16.4	17.5
Serine phosphatide	4.3	4.2
Choline phosphatide	60.0	61.2
Methanol eluate	3.3	1.8

Sex differences in fatty acid distribution were small compared with differences in distribution among lipid classes. Comparing the composition of lipid fractions (as shown in Table IV) with that of total flesh lipid (Table II), it is clear that the sterol esters contained much less 16:0 and more 20:5ω3; ethanolamine phosphatides contained less 16:0, less 20:5ω3, and more 22:6ω3; and serine phosphatides contained less 16:0, and 20:5ω3, and much more 18:0 than the average total lipid. These fractions, however, represent only a small proportion of the total lipid (20–25%) and it is not surprising that the choline phosphatides, which represented about 60% of flesh lipid, had a fatty acid composition similar to that found in total lipid, but containing slightly less 22:6ω3 and more 20:5ω3 and 16:0.

Both the ethanolamine and the choline phosphatides contained an unidentifed peak, eluted after 22:6ω3 from both GLC columns. This was first considered to be a C_{24} polyunsaturate on the basis of log plot calculations. Linko (1965) has reported a 24:4 fatty acid in Baltic herring, but the unidentified peak encountered in the present study did not change position on hydrogenation, so its structure remains unknown.

These results are remarkably similar to those obtained by Roubal (1967) in a study of the flesh lipids of Pacific cod (*G. macrocephalus*). In this species also, triglycerides were low in polyunsaturates and high in monoenes (with the rather surprising exception of 16:1 which was concentrated in the phosphatidyl choline fraction); ethanolamine and choline phosphatides were also similar in composition to those fractions in Atlantic cod (present results). Serine phosphatides from the Pacific cod were not analysed.

Analyses similar to these have been reported for tuna white muscle lipids (Shuster et al., 1964); menhaden muscle phospholipids (Froines et al., 1965); hake flesh phospholipids (de Koning, 1966); and for phosphatidyl cholines from trout muscle (Gray and MacFarlane, 1961) and from various other marine sources (Menzel and Olcott, 1964). Some features of fatty acid distribution among lipid fractions are common to the various species studied by

TABLE IV. Fatty acid composition of flesh lipid fractions (acids as percentages of total C_{12}–C_{24}).

	Sterol esters		Triglycerides		Ethanolamine phosphatide		Serine phosphatide		Choline phosphatide	
Peak	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
12:0	–	–	Trace	0.2	0.4	–	–	–	–	–
14:0	1.5	2.2	4.4	3.8	0.5	0.8	0.5	0.5	1.4	1.0
14:1 + Br 15:0[a]	0.4	0.2	0.3		Trace		0.1		Trace	Trace
15:0	0.5	0.6	0.3	0.7	0.1	0.3	0.3	0.3	0.3	0.7
15:1 + Br 16:0	0.5	0.3	0.5	0.2	Trace		Trace		–	Trace
16:0	8.4	7.9	14.1	15.7	8.8	12.0	8.0	9.4	23.9	25.2
16:1ω7[b]	4.6	5.2	12.0	12.3	1.7	2.4	1.4	2.1	4.2	4.1
16:2ω4	Trace	0.2	0.4	0.2	0.3	0.2	0.6	0.4	0.5	0.6
16:3 + Br 17:0	0.2	0.2	0.5	Trace	0.1	0.1	0.2	0.3	Trace	0.3
17:0	0.3	0.1	0.4	1.2	0.3	0.1	0.1	0.4	0.2	Trace
17:1 + Br 18:0	0.1	0.2	0.5	0.4	0.1	0.1	Trace	0.2	Trace	Trace
18:0	1.6	2.0	2.6	3.9	4.4	5.4	18.9	17.8	2.6	2.9
18:1ω9	10.0	10.6	22.4	26.2	14.4	17.9	14.4	16.1	12.6	10.9
18:2ω6	0.7	1.3	0.9	1.5	0.8	0.8	0.7	0.6	0.9	0.9
18:3ω6	0.2	0.1	Trace	0.2	Trace	0.2	0.2	0.4	–	0.1
18:3ω3	0.4	0.6	0.2	0.5	0.3	0.3	0.2	0.2	0.2	0.2
18:4ω3	0.2	0.3	1.2	1.4	0.3	0.1	Trace	0.3	0.3	0.1
20:1ω9	3.9	3.9	12.1	8.3	4.0	3.0	5.6	3.6	1.7	0.7
20:2ω6	0.1	0.1	Trace	–	0.2	0.1	Trace	0.1	Trace	–
20:3ω3	0.2	0.2	Trace	–	–	–	0.3	–	–	Trace
20:4ω6	1.4	4.0	0.5	1.1	1.6	3.1	2.9	4.0	2.4	4.7
20:4ω3	0.2	2.2	0.1	0.4	0.3	Trace	0.2	0.4	0.1	Trace
20:5ω3	27.5	25.9	7.3	8.9	10.7	11.0	7.5	8.0	19.4	20.0
21:5ω2	0.6	0.3	–	0.1	0.2	Trace	0.1	Trace	Trace	Trace
22:1ω11	3.6	1.9	11.4	6.2	0.3	Trace	0.5	Trace	0.4	Trace
22:5ω6	–	0.2	–	–	–	–	–	–	–	–
22:5ω3	2.2	2.5	0.4	Trace	1.9	0.9	1.7	1.1	1.6	0.5
22:6ω3	30.7	26.8	7.4	6.6	40.5	36.8	35.6	33.8	27.3	25.0
24:1	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace	Trace
Unknown	–	–	–	–	7.8	4.3	Trace	Trace	–	2.1
% saturates	12.3	12.8	21.8	25.5	14.5	18.3	27.8	28.2	28.4	29.8
% monoenes	23.1	22.3	59.2	53.6	20.5	23.7	22.0	22.2	18.9	15.7
% polyunsaturates	64.6	64.9	19.0	20.9	57.2	53.6	50.2	49.6	52.7	52.4
Unknowns	–	–	–	–	7.8	4.3	–	–	–	2.1
Palmitic/stearic acid ratios	5.25	3.95	5.4	4.0	2.0	2.2	0.42	0.53	9.2	8.7

[a]Br = Branched chain.
[b]Other isomers may be present (cf. Ackman et al., 1967).

these authors. It appears that "neutral" lipids (nonphospholipids) are relatively low in polyunsaturates and high in monoenes. The present results, and those of Roubal (1967) suggest, however, that triglycerides are especially low in polyunsaturates, and the relatively high levels quoted for some neutral

lipid fractions may be due to the presence of sterol esters rich in these fatty acids. The cephalin fraction (ethanolamine and serine phosphatides) is richer in polyunsaturates than is the phosphatidyl choline fraction, and, again considering the present results, it seems that the level of polyunsaturates may be higher in the ethanolamine than in the serine phosphatides. The major saturated fatty acid in the cephalin fraction is 18:0 (possibly localized in the serine phosphatides) but in choline phosphatide is 16:0.

Shuster et al. (1964) have pointed out that the palmitic:stearic (P/S) acid ratio (see Table IV) seems to be a specific fatty acid composition characteristic of individual marine lipids. In the fish studied by these authors, neutral lipids and phosphatidyl choline had high P/S ratios, and cephalins low P/S ratios. In their study of lipid hydrolysis in frozen stored North Sea cod, Olley and Duncan (1965) found that the free fatty acids accumulated after 6 months at −14 C had a P/S ratio higher than the "parent" phospholipid, though otherwise the composition was similar. This they interpreted as implying that phosphatidyl choline was the major source of free fatty acids, and confirmation of this came from the results of Bligh and Scott (1966). The present results show that cod flesh choline phosphatide has a fatty acid composition very similar to that found in free fatty acid by Olley and Duncan (1965). The slightly higher level of 22:6ω3 in free fatty acid might be attributed to minor differences in sample origin, but more probably to the fact that some of the free fatty acid arises from phosphatidyl ethanolamine (Bligh and Scott, 1966) which appears to be quite rich in this acid.

As stated above, the flesh lipids of cod and other lean fish probably do not represent an energy reserve. However, the basic similarity in fatty acid composition of individual phospholipids from various species of fish suggests that these compounds have a common function (or functions). The phospholipids as a group are believed to be involved in membrane structure (Davson and Danielli, 1943), electron transfer (Green and Fleischer, 1964), and experimental evidence has been obtained to show that lecithin is involved in the solution and concentration of enzymes (Jurtshuk et al., 1963). Although the significance of the fatty acid distribution in individual lipids is not yet understood, it should become clearer as the biological roles of the lipids are elucidated.

REFERENCES

ACKMAN, R. G., AND R. D. BURGHER. 1964. Cod flesh: component fatty acids as determined by gas–liquid chromatography. J. Fish. Res. Bd. Canada, **21**: 367–371.

1965. Cod liver oil fatty acids as secondary reference standards in the GLC of polyunsaturated fatty acids of animal origin: analysis of a dermal oil of the Atlantic leatherback turtle. J. Am. Oil Chemists' Soc., **42**: 38–42.

ACKMAN, R. G., AND J. C. SIPOS. 1964. Application of specific response factors in the gas-chromatography analysis of methyl esters of fatty acids with flame ionization detectors. Ibid., **41**: 377–378.

ACKMAN, R. G., J. C. SIPOS, AND P. M. JANGAARD. 1967. A quantitation problem in the open tubular gas chromatography of fatty acid esters from cod liver lipids. Lipids, **2**(3): 251–257.

BLIGH, E. G., AND W. J. DYER. 1959. A rapid method of total lipid extraction and purification. Canadian J. Biochem. Physiol., **37**: 911–917.

BLIGH, E. G., AND M. A. SCOTT. 1966. Lipids of cod muscle and the effect of frozen storage. J. Fish. Res. Bd. Canada, **23**: 1025–1036.

DAVSON, H., AND J. F. DANIELLI. 1943. The permeability of natural membranes. Cambridge University Press.

DE KONING, A. J. 1966. Phospholipids of marine origin. 1. The hake (*Merlucius capensis*, Castelnau). J. Sci. Food Agr., **17**: 112–117.

FROINES, J. R., C. Y. SHUSTER, AND H. S. OLCOTT. 1965. Phospholipids of menhaden muscle. J. Am. Oil Chemists' Soc., **41**: 887–888.

GARCIA, M. D., J. A. LOVERN, AND J. OLLEY. 1956. The lipids of fish. 6. The lipids of cod flesh. Biochem. J., **62**: 99–107.

GRAY, G. M., AND M. G. MACFARLANE. 1961. Composition of phospholipids of rabbit, pigeon and trout muscle and various pig tissues. Ibid., **81**: 480–488.

GREEN, D. E., AND S. FLEISCHER. 1964. Role of lipid in mitochondrial function. *In* R. M. C. Dawson and D. N. Rhodes [ed.] Metabolism and physiological significance of lipids. Wiley, London. p. 581–618.

HIRSCH, J., AND E. H. AHRENS. 1958. The separation of complex lipid mixtures by the use of silicic acid chromatography. J. Biol. Chem., **233**: 311–325.

JANGAARD, P. M., R. G. ACKMAN, AND J. C. SIPOS. 1967a. Seasonal changes in fatty acid composition of cod liver, flesh, roe and milt lipids. J. Fish. Res. Bd. Canada, **24**: 613–627.

JANGAARD, P. M., H. BROCKERHOFF, R. D. BURGHER, AND R. J. HOYLE. 1967b. Seasonal changes in general condition and lipid content of cod from inshore waters. Ibid., **24**: 607–612.

JURTSHUK, P., JR., I. SEKUZU, AND D. E. GREEN. 1963. Studies on the electron transfer system. LVI. On the formation of an active complex between apo-D(—)-β-hydroxybutyric dehydrogenase and micellar lecithin. J. Biol. Chem., **238**: 3595–3605.

LINKO, R. R. 1965. 12,15,18,21-Tetracosatetraenoic acid in the lipids of the Baltic herring. Suomen Kemistilehti, B**38**: 245.

LOVERN, J. A., J. OLLEY, AND H. A. WATSON. 1959. Changes in the lipids of cod during storage in ice. J. Sci. Food Agr., **10**: 327–337.

MENZEL, D. B., AND H. S. OLCOTT. 1964. Positional distribution of fatty acids in fish and other animal lecithins. Biochim. Biophys. Acta, **84**: 133–139.

MORRISON, W. R., AND L. M. SMITH. 1964. Preparation of fatty acid methyl esters and dimethyl acetals from lipids with boron fluoride–methanol. J. Lipid Res., **5**: 600–608.

OLLEY, J., AND W. R. H. DUNCAN. 1965. Lipids and protein denaturation in fish muscle. J. Sci. Food Agr., **16**: 99–103.

OLLEY, J., AND J. A. LOVERN. 1960. Phospholipid hydrolysis in cod flesh stored at various temperatures. Ibid., **11**: 644–652.

ROUBAL, W. T. 1967. Oxidative deterioration of flesh lipids of Pacific cod (*Gadus macrocephalus*). J. Am. Oil Chemists' Soc., **44**: 325–327.

SHUSTER, C. Y., J. R. FROINES, AND H. S. OLCOTT. 1964. Phospholipids of tuna white muscle. Ibid., **41**: 36–41.

SPITZER, H. L., AND J. A. BALINT. 1964. A rapid quantitative separation of phosphatidyl ethanolamine and phosphatidyl serine by column chromatography. Anal. Biochem., **5**: 143–148.

Growth and Reproduction of the Lake Whitefish, *Coregonus clupeaformis*, in Lac la Ronge, Saskatchewan[1]

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ABSTRACT

In Lac la Ronge the lake whitefish (*Coregonus clupeaformis* (Mitchill)) spawned from late October until the middle of November at ages of 8 years and over. The growing season lasted from late May to early October and young fish grew faster than older ones and tended to have a longer growing season. Ages for 1604 fish taken during 4 years showed that they grew to a fork length of about 16 inches in 10 years, the rate being slower than for lake whitefish in other large lakes in North America. The length–weight relationships were linear: for the open lake, log W = 3.48 log L – 3.93, and for Hunter Bay, log W = 3.26 log L – 3.66. The males matured a little earlier than females and their life span was somewhat shorter. The sex ratio was approximately 1:1.

INTRODUCTION

The lake whitefish (*Coregonus clupeaformis* (Mitchill)) fishery is of commercial importance in Lac la Ronge. To help elucidate the biology of this species in Lac la Ronge and other lakes in the Churchill River valley and on the Precambrian Shield of Saskatchewan, growth rate, stage of maturity, fecundity, sex ratios, and mortality rates were studied in both the eutrophic (main lake) and the oligotrophic (Hunter Bay) parts of the lake.

Lac la Ronge is situated in the central part of Saskatchewan and is made up of three natural divisions: the open shallow area (mean depth, 11 m) to the south, comprising about 160 sq miles; the northern islands area, about 290 sq miles (mean depth, 14 m); and Hunter Bay, the deepest region (mean depth, 20.7 m), about 50 sq miles. The open region and the islands region together are called the main lake. For location and limnology of this lake consult Rawson and Atton (1953).

MATERIALS AND GENERAL METHODS

This study was based on 14,443 lake whitefish collected on Lac la Ronge during the years 1948–50 and 1953–54. More than half the fish (9924) were sampled by a standard gang of gillnets as described by Rawson (1951). The rest were collected from the commercial catches in 5- or 5.5-inch stretched mesh nets.

Ages of 1034 lake whitefish from the main lake and 570 from Hunter Bay were determined according to the method outlined by Rawson and Atton

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(1953). The scales for age study were taken from an area below the anterior end of the dorsal fin of the whitefish; their impressions were made on cellulose acetate slides and magnified 45 times.

The differences between the fork lengths of the lake whitefish in the main lake and Hunter Bay were tested statistically by *t*-tests as prescribed by Burns (1966). The mean values of the fork lengths for ages 4–8 were all different ($p = 0.05$) (number of whitefish in parentheses):

Age (*years*)	Main lake		Hunter Bay		*t*-test	
	Length (*inches*)	Variance	Length (*inches*)	Variance	df	Calculated *t*
4	9.6(42)	2.688	9.1(24)	0.357	64	14.37
5	11.0(47)	0.311	9.7(24)	4.186	69	4.02
6	12.1(115)	0.329	11.2(36)	0.288	149	7.69
7	13.3(93)	0.261	12.7(37)	0.242	128	6.18
8	14.3(175)	0.671	13.8(36)	0.262	209	3.12

Number of whitefish collected between the ages of 1 and 3 was not adequate for this type of analysis. Whitefish of ages 9–19 were not considered for *t*-tests because some of them were caught commercially.

The weights of whitefish at various ages were not tested statistically.

Since significant differences were found in the fork lengths of younger age-groups between the main lake and Hunter Bay whitefish, all the data were treated separately for the two regions.

Sex and stage of maturity of whitefish were determined during the summers of 1948 (2049 fish, Table II) and 1954 (3058 fish), and spawning condition in October 1953 (102 fish). In late August and early September 1954, ovaries of eight fish from the open lake and four from Hunter Bay were collected and preserved in formalin to estimate fecundity. The total number of eggs in an ovary was estimated from the ratio between the volume of the ovary and the volume of the small portion of the ovary in which eggs were counted. The number of eggs in the portions of ovaries sampled ranged between 126 and 399.

For the year 1954, the relationships between the fork length and weight and fork length and anterior scale radius length were calculated for the open lake and Hunter Bay whitefish.

The seasonal growth was studied for the open lake and Hunter Bay whitefish from early June to early September in 1954. For this study the scales were magnified 50 times. The increase in the antero-posterior diameter of a scale beyond the last annulus was considered as a measure of the increase in the length of the whitefish between the time the last annulus was formed and the time of the capture of fish. This increment in the scale diameter was measured in millimeters. Three scales were measured from an individual fish and three to seven fish representing a 15-day period were used. However,

for a few 15-day intervals data were not available. The average increments in the scale diameters for 15-day periods were plotted against the 7th and 23rd days of each month. In determining the relation between scale length and body length, the anterior scale radius was used.

SPAWNING AND FECUNDITY

The spawning migration in the lake whitefish was observed in 1953; it took place between the 3rd and 4th weeks in October. In the open lake the fish migrated toward the south, southwest, east, and southeast shores, and some toward the mouth of the Montreal River. In the islands region most whitefish migrated to the north and northeast shores. In Hunter Bay they migrated to the north and east arms. The males reached the spawning grounds several days before females.

Spawning fish were 8 years old or older. No external anatomical change was noticed in the females. The males, however, became rough with nuptial tubercles on the head, cheek, and opercle, and also on the scales on and adjacent to the lateral line. During their spawning period, which lasted from late October to mid November, the fish swam off the bottom and stopped feeding. They were active, leaped out of the water, and splashed on the surface at sunset and during the night; spawning presumably occurred during the night. Similar observations on the spawning behaviour of lake whitefish were made by Hart (1930) and Van Oosten and Hile (1949).

Generally, egg production in whitefish increased with the increased size of a fish. Some of the smaller fish produced more eggs than larger ones. For the open lake, the mean weight of eight mature whitefish was 1.6 (1.3–2.2) lb; a mean number of 7155 (3972–9969) eggs per pound of fish weight was produced by these fish. For Hunter Bay, the mean weight of four mature whitefish was 2.6 (2.2–3.0) lb; these fish produced a mean number of 9018 (7948–11,200) eggs per pound of fish weight. The numbers of eggs produced were within the ranges reported for the species by earlier workers (Hart, 1930; Kennedy, 1953; Lawler, 1961).

INCREASE IN LENGTH AND WEIGHT

Length and weight at a given age were similar for males and females and from year to year. Therefore, the data for both the sexes and for all the years were combined and are summarized in Table I.

In the main lake younger whitefish increased in length slightly faster than those in Hunter Bay (Table I). About age-group 8 the growth rate in Hunter Bay was higher than in the main lake and beyond age 9 length of the fish in the Bay approximated that in the main lake. In the main lake and Bay, whitefish attained a fork length of 16 inches in 10 years.

In the main lake, during the first 8 years, the increase in weight of whitefish was slightly greater than that in Hunter Bay (Table I). Generally beyond that age, weight of whitefish was greater in Hunter Bay than in the main lake.

TABLE I. Mean fork lengths (inches) and mean weights (pounds) of lake whitefish from the two areas of Lac la Ronge. The data from standard gang samples for the years 1948, 1949, 1953, and 1954 and from commercial catches for 1948 and 1954 are combined.[a]

	Age (*years*)																		
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Main lake																			
Fork length	6.4	7.0	8.5	9.6	11.0	12.1	13.3	14.3	15.4	16.5	17.3	18.2	19.0	20.0	20.8	22.6	–	–	–
Wt	0.13	0.15	0.24	0.41	0.60	0.76	1.07	1.36	1.65	1.95	2.23	2.70	3.01	3.59	4.47	5.43	–	–	–
No. (1034)	4	30	48	42	47	115	93	175	228	127	59	35	18	7	3	3	–	–	–
Hunter Bay																			
Fork length	–	6.7	8.2	9.1	9.7	11.2	12.7	13.8	15.0	16.2	17.1	18.0	18.9	19.7	20.7	–	23.7	24.8	25.0
Wt	–	0.16	0.23	0.32	0.45	0.63	0.93	1.30	1.66	2.00	2.40	2.79	3.16	3.54	4.05	–	5.60	6.00	5.80
No. (570)	–	5	7	24	24	36	37	36	63	62	89	84	49	34	13	–	3	3	1

[a]Numbers of whitefish in Tables I and III do not correspond; in Table III whitefish caught commercially are not included; in Table I sex for some whitefish was not determined and these are not included.

The length–weight relationships were found to be linear: for the open lake whitefish, log W = 3.48 log L – 3.93, and for Hunter Bay whitefish, log W = 3.26 log L – 3.66.

The fork length in inches (Y) and the anterior scale radius (X), magnified 50 times and measured in millimeters, indicated a straight line relationship: Y = 0.959 + 1.123 X for the open lake and Y = 0.863 + 1.047 X for Hunter Bay. The scales on the whitefish in the open lake and Hunter Bay were formed at a fork length of 0.95 and 0.86 inches, respectively.

SEASONAL GROWTH

In the open lake, whitefish in their first 6 years resumed growth in late May or early June, with an increasing growth rate in June and July (Fig. 1). Age-groups 7–13 resumed their growth in the first half of June and growth rate was maximum in July. During the summer, growth tended to last for a longer period in younger fish than in the older fish. Beyond age-group 9, growth was slow. Growth in all age-groups decreased in August and September. In Hunter Bay, the growth pattern was slightly different. Age-groups 3–10 resumed growth in late May or early June, with maximum growth in late June and July. Beyond age-group 11, growth was slow and the maximum growth period was in late June and July. In late August and September, growth decreased.

Atton (MS, 1955) examined the effects of temperature on the growth of lake whitefish in Lac la Ronge and showed that for the open lake, 7% of the annual growth occurred in May, 26% in June, 46% in July, 17% in August, and 4% in September. In Hunter Bay, 5% of the annual growth took place in May, 21% in June, 41% in July, 28% in August, and 5% in September. The findings in the present study were similar to these except that in August there was no consistent difference in the percentages of the annual growth.

Kennedy (1953) showed that lake whitefish in Great Slave Lake grew from the middle of June to the end of September and that maximum growth was made during the first half of August. Kennedy (1943) also showed that lake whitefish in Lake Opeongo grew from May to September, and younger fish grew faster and for a longer period. Budd and Cucin (1962) mentioned that the growing season for Lake Huron lake whitefish lasted from June to the end of September.

SIZE AT MATURATION

In the main lake, a few male and female whitefish matured at a fork length of about 8 inches (Table II). At fork lengths 14.0–15.9 inches the percentage of the total that were mature reached a maximum for males and females, and the percentage of females that were mature was slightly higher than that of males. At fork lengths shorter than 14.0 inches the percentage mature was usually higher for males than for females, and at lengths greater than 16.0 inches the percentages were generally similar for males and females.

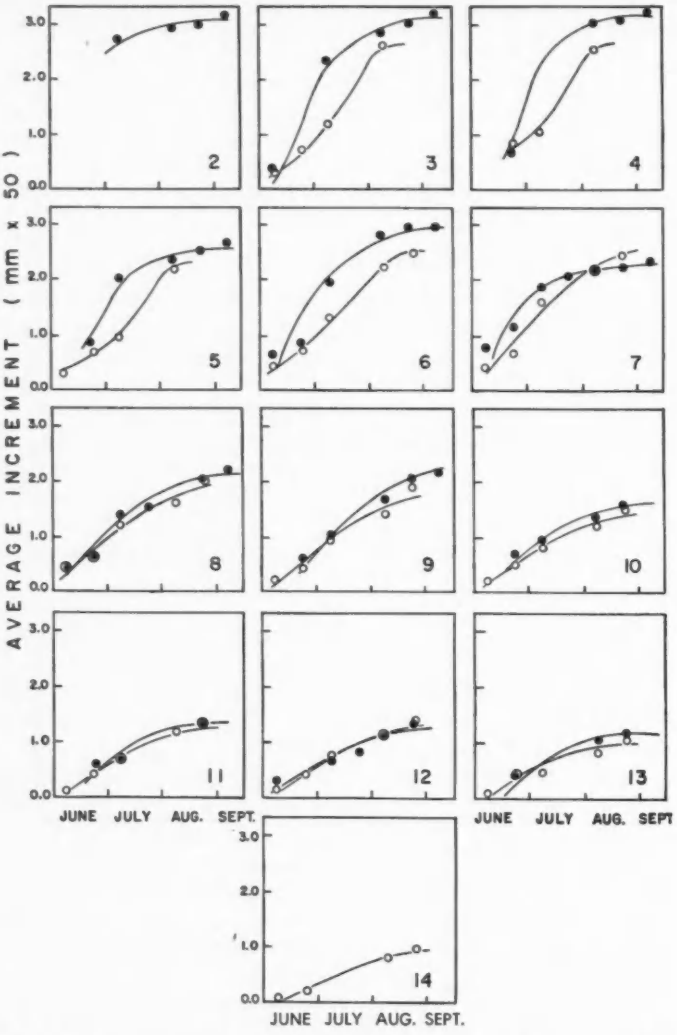

FIG. 1. Seasonal growth of lake whitefish of the various age-groups in the open lake (●) and in Hunter Bay (○) during the summer of 1954. The points represent the average increments (×50) in the antero-posterior diameter of a scale beyond the last annulus. The lines were fitted by eye.

For Hunter Bay, the sizes of samples were small and did not show the ratios conclusively. Van Oosten (1939) and Van Oosten and Hile (1949) observed that males attained maturity earlier than do females.

TABLE II. Percentages of male and female lake whitefish from the main lake and from Hunter Bay that were mature in the summer of 1948. Immature fish are not included.[a] Fish were collected with a standard gang of gillnets.

	Main lake (1820 fish)		Hunter Bay (229 fish)	
	Percentage mature		Percentage mature	
Length (*inches*)	Males (931 fish)	Females (889)	Males (115)	Females (114)
5.0–10.9	3.4	1.8	6.6	3.7
11.0–11.9	1.9	1.6	3.3	5.9
12.0–12.9	4.6	4.2	0.4	3.3
13.0–13.9	6.7	5.4	1.5	1.5
14.0–14.9	8.7	9.1	1.8	2.9
15.0–15.9	9.2	10.8	2.9	5.9
16.0–16.9	5.1	4.7	6.6	4.8
17.0–17.9	2.9	2.4	3.7	7.4
18.0–18.9	0.9	1.4	5.9	5.9
Over 19.0	0.6	0.7	9.5	0.7
Total	43.9	42.0	42.4	42.0

[a]In the main lake 14.0% of lake whitefish were immature and in Hunter Bay 15.6%.

SEX RATIO AT VARIOUS AGES

Little or no difference was observed between the percentages of males and females in whitefish samples from the main lake and Hunter Bay. The percentages in the samples for the years 1948 and 1954 combined were:

	No.	% male	% female	% immature
Main Lake	4312	46.0	43.3	10.7
Hunter Bay	795	45.2	46.6	8.2

Also, the samples from the above two regions did not show noticeable difference between the percentages of males and females in various age-groups (Table III).

Pearl (1916) found 45.9% males and 54.1% females in lake whitefish captured off Dunkirk, New York, in early July. The percentage of males varied from 43.2 to 48.3% during the 5 days of observations. Van Oosten and Hile (1949) showed the numbers of males and females in a nonspawning run of whitefish in Lake Erie were nearly equal. Kennedy (1953) examined 918 lake whitefish from Great Slave Lake and found that 451 were males and 467 females.

TABLE III. Age distributions (percentages) of male and female lake whitefish caught in standard gangs of gillnets in the two areas of the lake. Data for 1948, 1949, 1953, and 1954 are combined.

	Age (*years*)																
	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	Total
Main lake (978 fish)																
% males	1.3	2.1	2.1	3.1	4.8	5.2	9.3	12.6	6.2	2.6	1.3	0.8	–	0.2	–	51.7
% female	0.8	2.2	2.1	1.7	5.5	4.1	8.9	10.5	6.5	2.9	1.5	0.7	0.5	–	0.1	48.2
Hunter Bay (411 fish)																
% males	0.7	0.7	4.1	3.6	4.1	3.1	3.6	4.3	6.9	6.5	4.3	4.1	2.5	0.7	–	49.4
% females	0.2	0.5	1.7	1.9	4.3	5.8	5.3	5.1	6.7	6.5	6.9	3.4	0.7	0.5	–	49.6

ANNUAL MORTALITY RATES

Mortality rates were calculated according to the method outlined by Rounsefell and Everhart (1953). The fish used were caught in standard gangs of gillnets in 1953 and 1954, and the age distributions (percentages) were:

Age:	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	
Main lake (912 fish)	0.2	1.9	4.2	3.8	4.7	10.0	9.0	18.6	23.9	13.1	5.6	2.9	1.3	0.3	0.2	100%
Hunter Bay (361 fish)	–	0.6	1.7	5.3	6.1	8.9	9.1	9.7	10.5	14.9	14.1	9.7	5.5	3.1	0.8	100%

The average annual mortality rate of lake whitefish from the main lake between the ages of 9 and 13 years was 51.5% and for the Hunter Bay whitefish between the ages of 10 and 14 years 33.0%.

The above mortality rates were lower than 61.0% in the Great Slave Lake whitefish (Kennedy, 1953) and 64.0% in the Lake Winnipeg whitefish (Kennedy, 1954).

DISCUSSION

It is presumed that because of warmer water (Atton, 1955) and a richer food supply (Qadri, 1961) the young whitefish in the main lake grew faster than those in Hunter Bay. About the age of 6–7 years, increase in length of the Hunter Bay whitefish was greater than that in the main lake. After the age of 9 years length of the Hunter Bay whitefish approximated the length of whitefish in the main lake. Similar observations were made by Rawson (1961) on lake char (*Cristivomer namaycush*) from Lac la Ronge. He suggested that some change in feeding reversed the advantage and allowed the Hunter Bay lake char of about age 10 to grow faster than those in the main lake. Such an ecological change may have been one cause of accelerated growth in the Hunter Bay whitefish.

Comparisons of the rates of growth of lake whitefish in different areas of North America have shown that it grows faster near southern limits of its distribution (Van Oosten and Hile, 1949; Kennedy, 1954) and slower in the north (Rawson, 1947; Atton and Novakowski, MS, 1954; Kennedy, 1953 and 1963). The rate of growth of the lake whitefish in Lac la Ronge is close to that of the lake whitefish in Great Slave Lake where it attained a fork length of about 17 inches in 10 years. One would have assumed it to be intermediate between the growth rates found in whitefish from the northern and southern lakes.

The decrease in the relative abundance of males in the older age-groups (Table II) points toward a differential mortality of the sexes in nature, that is, females may have inherently a longer life span than males.

Since male lake whitefish mature at an earlier age than do females, attention must be given primarily to the females in the consideration of the proper size limit for the protection of the immature whitefish. The fact that the age-group 8 (14.0–14.9 inches in fork length) is the youngest age-group containing an appreciable number (although not a majority) of mature females suggests that at least this group should have full protection through their first spawning, at the end of their 9th year of life.

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REFERENCES

ATTON, F. M. MS, 1955. Thermal studies of Lac la Ronge, 1949 to 1952. M.A. Thesis. University of Saskatchewan, Saskatoon, Sask. 95 p.

ATTON, F. M., AND N. S. NOVAKOWSKI. MS, 1954. Analysis of sampling the commercial fishery of Lake Athabaska. Sask. Dept. Nat. Resources, MS Rept. 19 p.

BUDD, JOHN C., AND D. CUCIN. 1962. Exploitation of Canadian Lake Huron whitefish. Trans. Am. Fish. Soc., **191**: 223–224.

BURNS, JAMES W. 1966. Chi-square and the comparison of two means, *In* Inland fisheries management. Dept. Fish Game, State Calif. p. 162–167.

HART, J. L. 1930. The spawning and early life history of whitefish, *Coregonus clupeaformis* (Mitchill) in the Bay of Quinte, Ontario. Contrib. Canadian Biol. Fish., **6**: 165–214.

KENNEDY, W. A. 1943. The whitefish, *Coregonus clupeaformis* (Mitchill) of Lake Opeongo, Algonquin Park, Ontario. Univ. Toronto Studies, Biol. Ser., No. 51, Publ. Ont. Fish. Res. Lab., No. 62. p. 21–66.

1953. Growth, maturity, fecundity, and mortality in the relatively unexploited whitefish, *Coregonus clupeaformis*, of Great Slave Lake. J. Fish. Res. Bd. Canada, **10**: 413–441.

1954. Tagging returns, age studies, and fluctuations in abundance of Lake Winnipeg whitefish, 1931–1951. Ibid., **11**: 284–309.

1963. Growth and mortality of whitefish in three unexploited lakes in northern Canada. Ibid., **30**: 265–272.

LAWLER, G. H. 1961. Egg counts of Lake Erie whitefish. Ibid., **18**: 293–294.

PEARL, RAYMOND. 1916. On the proportion of sexes in whitefish. Mich. Acad. Sci., 17th Rept. (1915). 76 p.

QADRI, S. U. 1961. Food and distribution of lake whitefish in Lac la Ronge, Saskatchewan. Trans. Am. Fish. Soc., **90**: 303–307.

RAWSON, D. S. 1947. Estimating the fish production of Great Slave Lake. Ibid., **77**: 81–92.

1951. Studies of the fish of Great Slave Lake. J. Fish. Res. Bd. Canada, **8**: 207–240.

1961. The lake trout of Lac la Ronge, Saskatchewan. Ibid., **18**: 423–462.

RAWSON, D. S., AND F. M. ATTON. 1953. Biological investigation and fisheries management at Lac la Ronge, Saskatchewan. Sask. Dept. Nat. Resources, Fish. Rept. No. 1. 39 p.

ROUNSEFELL, GEORGE A., AND W. HARRY EVERHART. 1953. Fishery science, its methods and applications. John Wiley and Sons, Inc., New York.

VAN OOSTEN, JOHN. 1939. The age, growth, sexual maturity, and sex ratio of the common whitefish, *Coregonus clupeaformis* (Mitchill), of Lake Huron. Papers Mich. Acad. Sci., **24**(2): 195–221.

VAN OOSTEN, JOHN, AND RALPH HILE. 1949. Age and growth of the lake whitefish, *Coregonus clupeaformis* (Mitchill) in Lake Erie. Trans. Am. Fish. Soc., **77**: 178–249.

Nitrogen Fixation and the Utilization of Other Inorganic Nitrogen Sources in a Subarctic Lake[1,2]

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ABSTRACT

A year-round limnological study of the biological utilization of molecular nitrogen, ammonia, and nitrate in Smith Lake, a small subarctic lake in interior Alaska, showed that ammonia was consistently the most important nitrogen source. Of the two main algal production periods, the first took place under the ice in May, and depended on ammonia accumulated during the winter for a nitrogen source. The population at this time consisted largely of microflagellates. *Chlamydomonas*, *Euglena*, *Chlorella*, and *Mellamonas* were among the identified algae present. Immediately after the ice melted from the lake surface, a second population developed. These algae, consisting almost exclusively of *Anabaena flos-aquae*, used ammonia, nitrate, and molecular nitrogen simultaneously. During the remainder of the summer, uptake rates remained relatively low, with ammonia the most important nitrogen source; during the fall, nitrate uptake briefly approached the magnitude of ammonia uptake. ^{15}N tracer methods were used to measure the uptake rates in this work.

INTRODUCTION

In this paper, measurements of biological uptake rates of ^{15}N labeled ammonia, nitrate, and molecular nitrogen in a small arctic lake are discussed. The availability and utilization of nitrogen sources vitally influence, and perhaps at times control, the primary productivity of a lake. Since nitrogen undergoes a complex cycle in transport, very little can be assumed from information on the nutrient standing concentrations alone.

Measurements of instantaneous uptake rates of inorganic nitrogen nutrients have become possible using nitrogen compounds labeled with the stable isotope ^{15}N. In aquatic work, this technique was first applied to measurements of nitrogen fixation rates in fresh water. The method used was described in Neess et al. (1962), and the results of some of the first measurements are given in Dugdale and Dugdale (1962). In the latter paper, the importance of nitrogen fixation in the nitrogen nutrition of freshwater blue–green algae populations was confirmed. Previously, all observations of nitrogen fixation by algal blooms had been based on indirect evidence (see for example, Guseva, 1941; Utterback et al., 1942; Hutchinson, 1957).

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After this preliminary confirmation with ^{15}N, further work was carried out confirming nitrogen fixation in fresh water (Goering and Neess, 1964), in coastal regions (Stewart, 1964, 1965), and in the open sea (Dugdale et al., 1964).

The first freshwater measurements of the uptake rates of ammonia, nitrate, and molecular nitrogen have shown that all three sources may be used concurrently (Dugdale and Dugdale, 1965). This is contrary to the widely held opinion that nitrogen fixation is facultative, and unlikely to persist where adequate supplies of the energetically more easily utilizable nitrogen sources, nitrate and especially ammonia, are available. The present study, part of a broader study of the biological nitrogen cycle in lakes, was designed to provide information on the relative importance of the three major inorganic nitrogen sources, and to investigate their seasonal patterns of utilization.

METHODS

A single station was selected for sampling. The depth at the station was 2.5 m, and a permanent buoy was placed for sampling and incubation of samples. For the nitrogen uptake work, samples were taken manually with a 2000-ml polypropylene beaker. The beaker was immersed to a depth of about 0.25 m. Samples for nutrient chemistry and oxygen were taken with a 500-ml Frautschy bottle.

Samples for measuring the uptake of nitrate or ammonia were directly introduced into 1000-ml pyrex reagent bottles, and the ^{15}N labeled ammonium sulfate or potassium nitrate was added from ampoules using a disposable hypodermic syringe. Each ampoule contained 2 μg-at of nitrogen with a known ^{15}N enrichment of 95, 97.5, or 99 atom %. In general, a total amount of nitrogen tracer equivalent to about 10% of the concentration of that form of nitrogen in the water was added. The enrichment was kept at this low level to eliminate the possibility of stimulation to growth from the added nutrient.

The technique described in detail by Neess et al. (1962) was used for the nitrogen fixation experiments. The sample was placed in a pyrex round-bottomed flask, with a 24/40 standard taper top. The volume used was either 500 or 1000 ml depending on the density of the organisms in the water. The gases in the water were removed by flushing with a 80–20 helium–oxygen mixture at slightly reduced pressure. This was accomplished by attaching the flask to a manifold via a specially designed sparger unit. After the sparging treatment, the manifold was evacuated and ^{15}N enriched nitrogen gas was added from a reservoir by displacement with a H_2SO_4–Na_2SO_4 confining fluid. This restored the pressure in the flask to atmospheric levels.

Incubation was carried out in situ by suspension from the sampling buoy and, during the 2nd year, also under standard light conditions in a constant temperature room at 20 C during the summer and 5 or 10 C during the winter, such that the in situ temperatures were approximated. Incubation times of 4 hr were used.

The post-incubation procedure consisted of filtering the water through a Hurlburt glass filter (984-H ultra filter; Reeve, Angel and Co.), after which the filter was dried. Mass spectrometry was carried out using a Bendix Time-of-Flight mass spectrometer (Model 17-210). The inlet system of this machine was connected via a liquid nitrogen trap to a modified Coleman Nitrogen Analyzer. With this system, conversion of the sample to nitrogen gas was carried out using the modified Dumas procedure described by Barsdate and Dugdale (1965). The mass 28 and mass 29 peaks were scanned on the mass spectrometer, and an Oskar K chart reader was used to transfer the peak heights to IBM cards. The following calculations were carried out on an IBM 1620 computer. The isotope ratios calculated from the mass 29 and mass 28 peaks were converted into atom percent values according to the formula:

$$A_f = \frac{100\,r}{2 + r}$$

where A_f is the atom percent ^{15}N of the particulate material at the end of the incubation period. From this, the normal atom percent of unenriched nitrogen was subtracted. This value was determined by running isotope ratio determinations on unenriched filtered lake water.

The atom percent of the nitrogen supplied at the beginning of the experiment had to be known in order to calculate the amount of nitrogen taken up. In the case of nitrogen fixation experiments, in which all the unlabeled nitrogen was removed, it was simply the enrichment in the supplied gas. For the nitrate and ammonia uptake experiments, the amount of unlabeled nitrate and ammonia in the water had to be included in the calculation. The atom percent of the available nitrogen at the beginning of the experiment was designated A_1. The amount of nitrogen taken up during the experiment was then:

$$N_f = \frac{N_t \times A_f}{A_i}$$

where N_t is the total particulate nitrogen in the sample. This method of calculation is valid as long as the change in N_t during the experiment is very small. In practice, the change in N_t during a short-term experiment seldom exceeds 1%.

Particulate nitrogen was determined using a Coleman Nitrogen Analyzer on samples collected on Hurlburt glass filters. Chlorophyll *a* was determined spectrophotometrically after 24-hr dark extraction of the pigment in 90% acetone; a Beckman DB scanning spectrophotometer was used, and a method of calculation was adapted using the specific absorption for Chlorophyll *a* given by Bruinsma (1961):

$$\frac{\text{O.D. } 658 \times 14.9 \times \text{solvent volume}}{\text{Lake water volume filtered}} = \text{mg liter}^{-1}\text{Chl. } a.$$

The optical density at 750 mμ was first subtracted from the optical density at 658 mμ. The results obtained were a little lower than those obtained by Bruins's calculation method for total chlorophyll for the same water sample.

Nitrate was measured using a modification of the Mullin and Riley procedure (1955) similar to that described by Strickland and Parsons (1960). Internal standards were necessary for each sample, since the water color was highly variable and affected the sensitivity of the reduction step; small portions of a standard curve were run using the sample itself rather than relying on a distilled water standard curve. In addition, a 10-fold increase in the copper catalyst was required for the reduction of these samples, since the colored substance apparently complexed much of the added copper. For ammonia, the sodium phenate method of Riley (1953) was used after vacuum distillation or diffusion. The oxidation method of Richards and Kletsch (1964) was used for a few of the later analyses.

Oxogen was determined using the Alsterberg modification of the Winkler procedure, and *p*H was measured on a Beckman expanded-scale *p*H meter. Samples of water for phytoplankton identification were preserved using a modified Lugol's solution described by Verduin (1960). Membrane filter techniques were used to make rough quantitative estimates.

DESCRIPTION OF THE LAKE

Located in the Tanana Valley in interior Alaska, at a latitude of 64°52″N and 146°52″W, Smith Lake has a surface area of somewhat less than 40 acres, a maximum depth of 3.5 m, and a mean depth of 2.0 m. It is located on a wildlife study area owned by the University of Alaska, and is therefore not subject to extensive recreational use. Some areas of the watershed are under cultivation, and it is possible that fertilization of this land may affect the nutrient content of the lake during the time of year when there is considerable runoff. The lake lies in a depression in a forested region which is dry during the summer, but is essentially a marsh during the spring-thaw period. There is no permanent inlet, but during the spring thaw, much of the water enters through two distinct streams. There is a single outlet, also functional only in the spring.

The lake water shows a strong brown color, which is characteristic of some arctic and subarctic lakes. The responsible substance in the lake appears to be similar to the yellow-brown material described by Shapiro (1964). It is very stable, not readily precipitated, and absorbs light strongly in the shorter visible wave lengths.

The climate of the region is continental subarctic with extremely cold winters and rather warm summers and an abrupt transition between the seasons, especially in spring. The period of ice-cover lasts from mid-November to the end of May, the ice thickness reaching a maximum of 1.5 m, with a snow cover up to a thickness of 0.5 m.

SEASONAL VARIATIONS IN NITROGEN UPTAKE AND PHYTOPLANKTON POPULATIONS

The first population of phytoplankton to develop in spring followed a long winter period without any evidence of such organisms in the water. This initial population accumulated under the ice, appearing initially in the "deep" water adjacent to the mud surface (2.5 m), and was first detected by rapid increases in the chlorophyll and particulate nitrogen contents of the water in May (Fig. 1). The nitrogen uptake experiments showed that this population predominantly used ammonia for a nitrogen source, with some detectable nitrate uptake (Fig. 2). The organisms present were dominated by

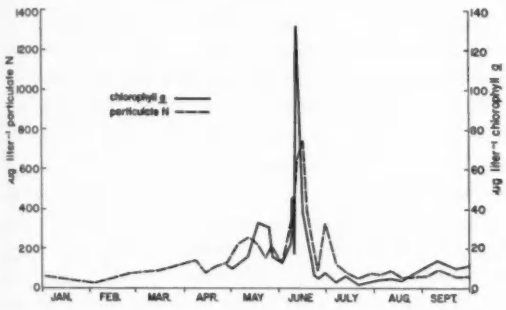

FIG. 1. Chlorophyll *a* and particulate nitrogen concentrations, Smith Lake surface water, 1964.

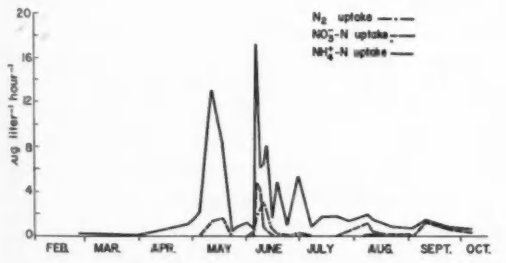

FIG. 2. Uptake rates for three inorganic nitrogen sources, Smith Lake surface water, 1964.

micro-flagellates. *Euglena*, *Chlamydomonas*, *Mellamonas*, and *Chlorella* were among the positively identified forms. Most of the smaller cells were not identified. The rate of ammonia removal at the peak of this growth was 13 μg liter^{-1} hr^{-1}. The amount of ammonia available in the water at the beginning of the bloom was 100.53 μg liter^{-1}. Ultimately, both the standing concentration of ammonia in the water and the ammonia uptake rate declined until no ammonia remained in the water. An excess of 133 μg liter^{-1} day^{-1} of ammonia was used over the amount apparently available, and the peak of the bloom coincided with the time when most of the ammonia had moved

into the particulate fraction. Concentrations of ammonia in the water during this bloom and the succeeding population peaks were:

Date:	April 14	May 13	May 23	May 27	June 8	June 10	June 25	July 13
Concentration (μg liter^{-1})	449.96	100.52	43.82	0.00	9.10	30.10	230.58	80.78

In 1964, the ammonia content of the runoff water which entered the lake through the temporary inlet ranged from 112.7 μg liter^{-1} on May 5 to 92.2 μg liter^{-1} on May 18. By May 27, it had declined to 27.6 μg liter^{-1}. Although this was an additional source of ammonia for the under-ice population, there was only a small volume of water entering the lake during the early part of May. By the end of May, when inflow increased, the ammonia content of this became low, and this source seemed unlikely to compete in importance as an additional nitrogen source with biological regeneration of ammonia within the lake itself.

In June, a spectacular bloom of *Anabena flos-aquae* followed the melting of the ice, and occurred during the period of rapid warming and increase in day length which culminated in a daylight period of approximately 24 hr on June 21. This population was concentrated near the surface. At this time, both the particulate nitrogen and the chlorophyll contents of the water reached their maximum peaks for the year (Fig. 1). This *Anabaena* population accounted for almost all the nitrogen fixation which took place during the year in this lake. The particulate nitrogen content (a useful approximation of the cell nitrogen content) and the nitrogen fixation rate increased simultaneously. The particulate nitrogen peak corresponded with the peak in the almost unialgal population of *Anabaena*, which was associated with nitrogen fixation rates (Fig. 3) approaching 1 μg liter^{-1} hr^{-1}. In spite of this nitrogen fixation, ammonia remained quantitatively many times more important as a nitrogen source and the uptake of nitrate also exceeded the uptake of molecular nitrogen (Fig. 2). There was very little ammonia present at the beginning of the *Anabaena* bloom. Most of the nitrogen was probably being released from the organic nitrogen fractions, both dissolved and particulate, with some cycling of newly assimilated and newly fixed nitrogen.

The cause of the abrupt termination of the bloom, even more extreme in 1964 than in 1963, is unclear. A spectacular increase in *p*H from the normal 7 to a value above 10 preceded the decline in both years, followed by restoration of normal *p*H within a day or two. This increase was probably caused by the extremely high photosynthetic rates during this time. The algae started to clump and form akinetes, and then disintegrated within a day or two. There may have been a relationship between bloom decline and *p*H increase; several mechanisms are possible. Nitrogen fixation requires a rather high supply of trace metals which are not essential or are needed in smaller amounts for growth on other nitrogen sources. Among these are molybdenum and iron.

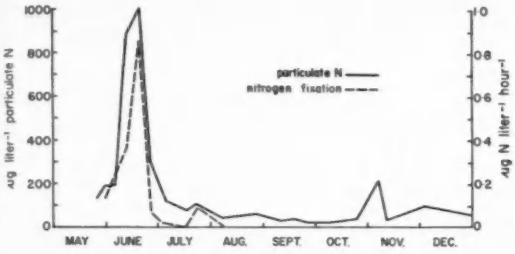

FIG. 3. Particulate nitrogen concentrations and nitrogen fixation rates in Smith Lake surfaces water, 1963.

In waters containing a high complement of colored material, the availability of iron has been shown to decrease with an increase in *p*H (Shapiro, 1964). The reduced availability of copper even at normal *p*H values was mentioned in the methods discussion above, and therefore it seems possible that this may have been an effective mechanism. Although an inadequate supply of CO_2 at high *p*H values could easily have limited the rate of photosynthesis, it seems less likely to have caused abrupt termination of a bloom. However, the response to any environmental deficiency may be the production of akinetes and the initiation of a dormant stage. The autotoxicity or autoinhibition of a dense population of blue–green algae can not be discounted, but there is insufficient information on the possibilities of such a mechanism.

During the remainder of the summer, ammonia remained the most significant source of nitrogen, with a relatively constant low concentration of this nutrient in the water, and relatively low uptake rates. Several minor populations of phytoplankton were found during this period, with *Aphanizomenon* predominant during much of the summer. A few *Anabeana* filaments were found and *Selenastrum* was another frequently found form. Zooplankton increased both in size and number during the summer, and grazing probably controlled the production of phytoplankton during July and August. In the fall, a brief pulse of algae, dominated by *Gymnodinium*, was found. During this period, nitrate uptake became quantitatively equal to ammonia uptake. This fall bloom was of minor significance compared with either of the spring pulses.

FACTORS CONTRIBUTING TO THE AVAILABILITY OF NITROGEN SOURCES

Events under the ice during the winter appeared to be important in determining the forms of nitrogen available to the phytoplankton in the spring. During the fall, the ammonia content of the water increased, accompanied by a similar increase in the nitrate concentration (Fig. 4). This was certainly due to the regeneration of ammonia from the organic nitrogen fractions, and its subsequent oxidation to nitrate by the bacterial process of nitrification. However, by early spring, the oxygen content of the water began to decline, ultimately resulting in anoxic conditions under the ice cover.

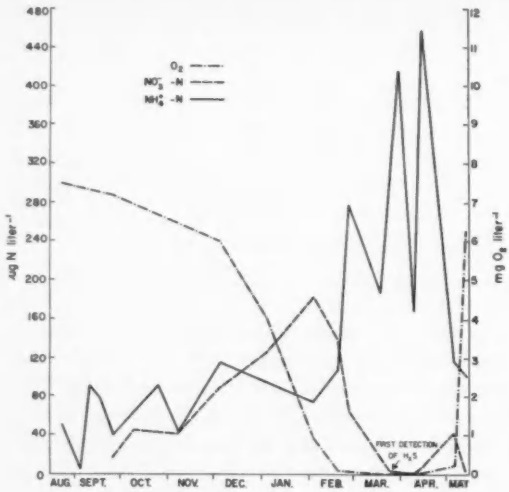

FIG. 4. Ammonia, nitrate, and dissolved oxygen concentrations in Smith Lake water under the ice, 1963–64.

As the oxygen content reached a low level, nitrate began to decrease in concentration until it disappeared completely. Hydrogen sulfide appeared in the water (detectable by odor) after the nitrate became exhausted.

The disappearance of nitrate can be attributed to dentrification, the bacterial dissimilatory reduction of nitrate, for the following reason. The reduction in nitrate concentration was occurring at a time when the oxygen content of the water was very low, and it is under these conditions that nitrate is used as a hydrogen acceptor by microorganisms, and reduced to nitrogen gas. ^{15}N tracer measurements have confirmed such reduction of nitrate to nitrogen gas in Smith Lake water at a rate adequate to account for the observed loss (Goering and Dugdale, 1966). As the nitrate concentration became low, sulfate took over a similar role, resulting in the accumulation of hydrogen sulfide.

Assuming that all the nitrate lost was denitrified, we can estimate a rate of dentrification in the water during this anoxic time from the rate of decrease in its nitrate content. If the vertical distribution of nitrate is plotted (for several dates) during this time (Fig. 5), the areas between the depth distribution lines represent the nitrate content for the water column, and, after integration of these areas, rates of nitrate loss can be determined from the differences.

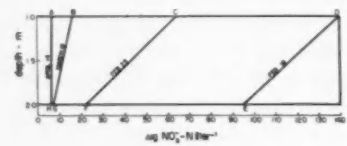

FIG. 5. Vertical distributions of nitrate in Smith Lake water, spring, 1964.

The total amount of nitrate lost during the spring amounted to 106.4 μg liter^{-1}, of which 74.2 μg liter^{-1} was lost during the first anoxic week.

Ammonia continued to increase in concentration in the water during this entire spring period. A high ammonia content was thus available to the phytoplankton in the spring, but very little nitrate.

GENERAL DISCUSSION

This seasonal work on nitrogen uptake in Smith Lake strongly supports the observation on Sanctuary Lake that ammonia is used by the population as a whole simultaneously with nitrogen supplied by nitrogen fixation (Dugdale and Dugdale, 1965). The proportions of ammonia, nitrate, and molecular nitrogen used in June were very similar in Sanctuary Lake to those shown for Smith Lake in Fig. 2. These conclusions can be applied only to the whole system, since no information obtained by the methods used applied to specific organisms within the population. However, the blue–green alga *Anabaena flosaquae* showed the most spectacular growth rate and abundance during the nitrogen-fixing phase.

The *Anabaena* bloom was observed using the methods described above during the 1963 and 1964 seasons, but was also noted during 1965, 1966, and 1967. The blooms were very similar in timing during these years.

A second conclusion, which emerges both from the information on the under-ice bloom and from the blue–green algal bloom, relates to the role of ammonia supply from other nitrogen fractions during a phytoplankton pulse. During both production periods, the amount of ammonia used was considerably in excess of the amount apparently available in the water. The ammonia content of the water actually increased during the *Anabaena* bloom. A steady rate of supply from the organic fraction is implied, and the appearance of ammonia in the water after the decline of the June bloom confirms this. The standing amount of ammonia in the water is, of course, determined by the difference between the rates of supply and removal. The significance of the instantaneous ammonia content in the water is thus of limited value in determining the nutrient available, unless the turnover rate of such ammonia is also known. Evidence supporting high ammonia turnover rates during algal blooms is presented in Billaud (1967). A very high concentration of ammonia, as found in the water prior to the under-ice bloom in May, however, suggests the potential for production.

The reappearance of oxygen in the water after the spring anoxic period coincided with the May algal production, and these organisms may have been responsible for producing the oxygen photosynthetically. By this time, there was considerable light, and penetration through the ice and snow could have been adequate. There was no evidence of algal production during the dark season, and therefore, no apparent need to postulate any important role for heterotrophy in the development of the under-ice bloom.

ACKNOWLEDGMENTS

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REFERENCES

BARSDATE, R. J., AND R. C. DUGDALE. 1965. Rapid conversion of organic nitrogen to N_2 for mass spectrometry: An automated Dumas procedure. Anal. Biochem., **13**: 1–5.

BILLAUD, V. A. (DUGDALE). 1967. Aspects of the nitrogen nutrition of some naturally occuring populations of blue-green algae. *In* Environmental requirements of blue-green algae. Proc. Symp. Sept. 23–24, 1966. U.S. Dept. Interior Federation Water Pollution Control Admin.

BRUINSMA, J. 1961. A comment on the spectrophotometric determination of chlorophyll. Biochem. Biophys. Acta, **52**: 576–578.

DUGDALE, V. A., AND R. C. DUGDALE. 1962. Nitrogen metabolism in lakes II. Role of nitrogen fixation in Sanctuary Lake, Pennsylvania. Limnol. Oceanog., **7**: 170–177.

1965. Nitrogen metabolism in lakes III. Tracer studies of the assimilation of inorganic nitrogen sources. Ibid., **10**: 53–57.

DUGDALE, R. C., J. J. GOERING, AND J. H. RYTHER. 1964. High nitrogen fixation rates in the Sargasso Sea and the Arabian Sea. Ibid., **9**: 507–510.

GOERING, J. J., AND V. A. DUGDALE. 1966. Estimation of rates of denitrification in a subarctic lake. Ibid., **11**: 113–117.

GOERING, J. J., AND J. C. NEESS. 1964. Nitrogen fixation in two Wisconsin lakes. Ibid., **9**(4): 530–539.

GUSEVA, K. A. 1941. [Bloom on the Ucha Reservoir.] Tr. Zool. Inst. Acad. Nauk SSSR, **7**: 89–121. (Tech. transl. 939, National Research Council of Canada.)

HUTCHINSON, G. E. 1957. A treatise on limnology. Vol. I. Chemistry, physics and geography. John Wiley, New York. 1015 p.

MULLIN, J. B., AND J. P. RILEY. 1955. The spectophotometric determination of nitrate in natural waters with particular reference to sea-water. Anal. Chim. Acta, **12**: 464–479.

NEESS, J. C., R. C. DUGDALE, V. A. DUGDALE, AND J. J. GOERING. 1962. Nitrogen metabolism in lakes. I. Measurements of nitogen fixation with ^{15}N. Limnol. Oceanog., **7**: 163–169.

RICHARDS, R. A., AND P. A. KLETSCH. 1964. The spectophotometric determination of ammonia and labile amino compounds in fresh and sea water by oxidation to nitrate. *In* Recent research in the fields of hydrosphere, atmosphere and nuclear geochemistry. Ed. Comm. Sugawara Festival Volume, Maruzen Co. Ltd., Tokyo. p. 65–81.

RILEY, J. P. 1953. The spectophotometric determination of ammonia in natural waters with particular reference to sea water. Anal. Chim. Acta, **9**: 575–589.

SHAPIRO, J. 1964. Effect of yellow organic acids on iron and other metals in water. J. Am. Water Works Assoc., **56**(8): 1062–1082.

STEWART, W. D. P. 1964. Nitrogen fixation by myxophyceae from marine environments. J. Gen. Microbiol., **36**: 415–422.

1965. Nitrogen turnover in marine and brackish habitats. I. Nitrogen fixation. Ann. Botany, London, **29**: 229–239.

STRICKLAND, J. D. H., AND T. R. PARSONS. 1960. A manual of seawater analysis. Bull. Fish. Res. Bd. Canada, No. 125. 185 p.

UTTERBACK, C. L., L. D. PHIFER, AND R. J. ROBINSON. 1942. Some chemical, physical and optical characteristics of Crater Lake. Ecology, **23**: 97–103.

VERDUIN, J. 1960. Phytoplankton communities of western Lake Erie and the CO_2 and O_2 changes associated with them. Limnol. Oceanog., **5**(4): 371–380.

Annulus Formation on Scales of Four Species of Coregonids Reared Under Artificial Conditions[1,2]

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ABSTRACT

Scales from known-age coregonids reared in the laboratory were examined to determine when annuli formed and to learn possible factors of their formation. Scales were taken monthly from marked fish for periods up to 21 months. Scales were also examined from fish that died and from preserved specimens of young-of-the-year for each species. Two marks formed on almost all scales each calender year. The stronger formed during March–April and the weaker in October–November. Both marks had all the usual characteristics of an annulus but the spring mark was considered the annulus and the fall mark an accessory check. The annulus formed during a period of constant temperatures and of little change in growth or increasing growth. The accessory check formed during a period of declining temperatures (1–5 degrees F, or 0.6–2.8 degrees C, per month) and of little change in growth or declining growth. Most fish grew throughout the winter; the only exceptions were one bloater (*Coregonus hoyi*) and several of the largest lake whitefish (*C. clupeaformis*). Fish were always given all the food they would eat to eliminate availability of food as a factor of mark formation. The temperature of the water during the winter (50 ± 0.3 F; 10.0 ± 0.2 C) did not arrest metabolic activity. The growth rate was related more closely to day length than to other variables examined.

INTRODUCTION

This study is based on scales from known-age coregonids (lake whitefish and ciscoes) reared at the U.S. Bureau of Commercial Fisheries Biological Station at Northville, Michigan. The study was initiated to determine if the fish formed annuli, and to find possible relationships between annulus formation and environmental conditions.

Van Oosten (1923) established the time of annulus formation for lake whitefish (*Coregonus clupeaformis*) reared in aquariums and later (1929) made a critical evaluation of the scale method for age determination of the lake herring (*C. artedi*) of Lake Huron. The basic assumptions inherent in the scale method and the views on factors of annulus formation developed by Van Oosten are mostly held valid today. The present study indicated, however, that length of day, not previously thought to influence annulus formation, may be an important factor for the coregonids.

Life histories have been studied for at least one population of most species of coregonids of the Great Lakes, but few of the studies established the time

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of annulus formation and none included experimental data on the formation of the marks used to estimate age and calculate growth. Evidence is provided here which emphasizes the need for careful validation of the year-mark on some populations of wild coregonids.

MATERIALS AND METHODS

The three species and one hybrid investigated were lake whitefish, bloater, kiyi (*C. kiyi*), and the first generation of a cross between female lake herring and male bloater.

In June or early July 1962, the largest fish in each of nine tanks, each containing various year-classes and species, was anesthetized, inoculated with antibiotics, measured, and injected with experimental orange dye (composition unknown to me). In September 1962, three young-of-the-year whitefish were similarly marked. Each month (with few exceptions) one or two scales were taken from the left side of each marked fish, between the dorsal fin and lateral line. If a marked fish died, the next largest fish in that tank was marked and a new series was started.

Scales were taken from 16 marked fish, including at least two individuals of each species and the hybrid (Table I). The number of scale samples from individual fish ranged from 3 to 17 over periods up to 21 months. A total of 137 scale samples from the marked fish was examined.

Scale samples were also obtained from unmarked fish that shared the tanks with the marked ones. When a fish died it was measured, labeled, and preserved. Scales for study were removed from two or three fish of each species that died each month through 1963. Scales from young-of-the-year of each species were also examined to determine scale development before the first annulus. A total of 288 scale samples from unmarked fish was used.

A correction factor of 1.023 was used to adjust the lengths of preserved fish before the regression equations of the body-scale relation used for back calculation of lengths were computed. This factor was determined by measuring 38 lake whitefish (250–400 mm long), 105 bloaters (100–250 mm), and 49 hybrids (100–300 mm) on the date of death and after preservation in 10% formalin for 1–3 years, when shrinkage had stabalized (Parker, 1963). Marked fish were measured alive at time of scale removal.

Cellulose acetate impressions were made of all scales (Smith, 1954) and examined at a magnification of 41× on a microprojector (Moffett, 1952). Two to five scales were examined from each unmarked fish and one or two scales for each date of removal from the marked fish. Application of several drops of acetone to the plastic strip, before impressing the scales, increased the clarity of the magnified image. Designation of age-groups followed the conventional practice which assumes that a fish becomes 1 year older on January 1 (Hile, 1936).

All fish were always fed as much as they would eat and all ate throughout the year.

TABLE I. Numbers of scale samples taken from various species of marked coregonids and periods of collection.

Species and year-class	Specimen no.	Period of collection	No. of months lived after marking[a]	No. of scale samples taken
Lake whitefish				
1962	1	9/14/62–5/1/63	8	4
1962	2	9/17/62–1/27/64	17	12
1962	3	9/17/62–12/10/63	16	9
1962	4	7/3/63–1/24/64	7	7
1959	5	6/8/62–12/10/63	19	17
1958	6	6/8/62–1/25/63	8	7
1958	7	6/8/62–2/28/64	21	16
Bloater				
1961	8	6/11/62–4/8/63	11	10
1961	9	7/5/63–3/7/64	9	8
1960	10	7/9/62–5/28/63	11	12
Lake herring × bloater hybrid				
1961	11	6/11/62–9/14/62	4	3
1961	12	6/13/62–1/15/63	8	7
1961	13	10/15/62–7/5/63	10	8
1961	14	8/9/63–2/6/64	7	6
Kiyi				
1961	15	6/8/62–4/8/63	11	8
1961	16	7/10/62–9/17/63	15	13
Total				137

[a]Includes months of marking and death.

The temperature of the spring water at the Northville station was 50 F (10 C). The water was sprayed into a large cement raceway and then pumped to the rearing troughs and tanks, or was taken by gravity feed from a large holding pond. Daily water temperatures for each tank were averaged by 10-day intervals.

A detailed account of methods, data, and analyses is given in the thesis upon which this paper is based (Hogman, MS, 1965).

TIME OF ANNULUS FORMATION

Almost all the scales had two strong marks each year, more than could be accounted for as annuli. The diameter increments within the two most recent marks and the total anterior-posterior diameter on each scale were measured along an imaginary line that passed through the focus and bisected the anterior and posterior fields (Van Oosten, 1929). After the scales of all groups of fish had been examined twice (randomly for each species) the percentage increase in scale diameter beyond the last mark was calculated for scales collected each month. The percentage increases for each fish were then arranged chronologically. Check formation was indicated when the percentage

increase beyond the outermost mark dropped suddenly and then began to increase again.

A check that represented the true annulus (Fig. 1A, B) began to form on all species in late March 1963, and had usually circled the scale by mid-April. The earliest indication of annulus formation was on March 12, 1963, for a age-I whitefish. Annulus formation was not complete for some fish longer than 250 mm until late April. Fish entering their second through fourth growing seasons (age I–III) formed annuli slightly earlier than seven marked specimens age IV and older examined at or just after annulus formation.

All unmarked fish over 1 year old had the same pattern of dual marks on their scales for all years of life. The percentage increases in scale diameter beyond the outer mark could not be averaged for the unmarked fish because of the small number (1–3) available (that had died) each month for each species; and because of the great differences in growth of individual fish. However, direct observation of scales from unmarked fish collected before and after annulus and accessory check formation of the marked fish, indicated that they also formed their annulus in late March – early April and an accessory check in late October – early November.

Scales from fish that had ceased growing or were growing very slowly preceding annulus formation (three large whitefish and two bloaters) suggested that scale growth resumed before a measurable increase was detectable in the total length. The data did not permit evaluation of the relation of size within an age-group to time of annulus formation.

To determine the validity of annulus identification and provide further evidence for time of occurrence, the lengths of the marked fish were back-calculated for the 1962 and 1963 annuli and compared with known lengths at various dates. This method indicated that the 1962 annulus formed in March or April. The identification of the 1963 annulus, for all scales collected after April, was confirmed in like manner. Although fish were not marked until June 1962, they were measured monthly beginning in March 1962. By comparing the average back-calculated length (at the 1962 annulus) with the size of the largest fish in the tank for the months before June 1962, the March–April formation of the 1962 annulus was confirmed. The marked fish always maintained their size advantage over others in the tank after June 1962; therefore the assumption that they were also the largest during March to June seems warranted. A retention of size advantage has been demonstrated for brown trout (*Salmo trutta*) reared under artificial conditions (Brown, 1946).

The number of annuli on each fish corresponded to its age. Many fish exhibited highly distinct annuli (Fig. 1A and 3A), but most scales were more difficult to interpret. Often the year-mark was so faint it might have been overlooked on wild fish of unknown age.

TIME OF ACCESSORY CHECK FORMATION

An accessory check (Fig. 1, 3) was formed in the fall on scales of nearly all fish studied. Formation of the mark began in mid-October and was complete by mid-November.

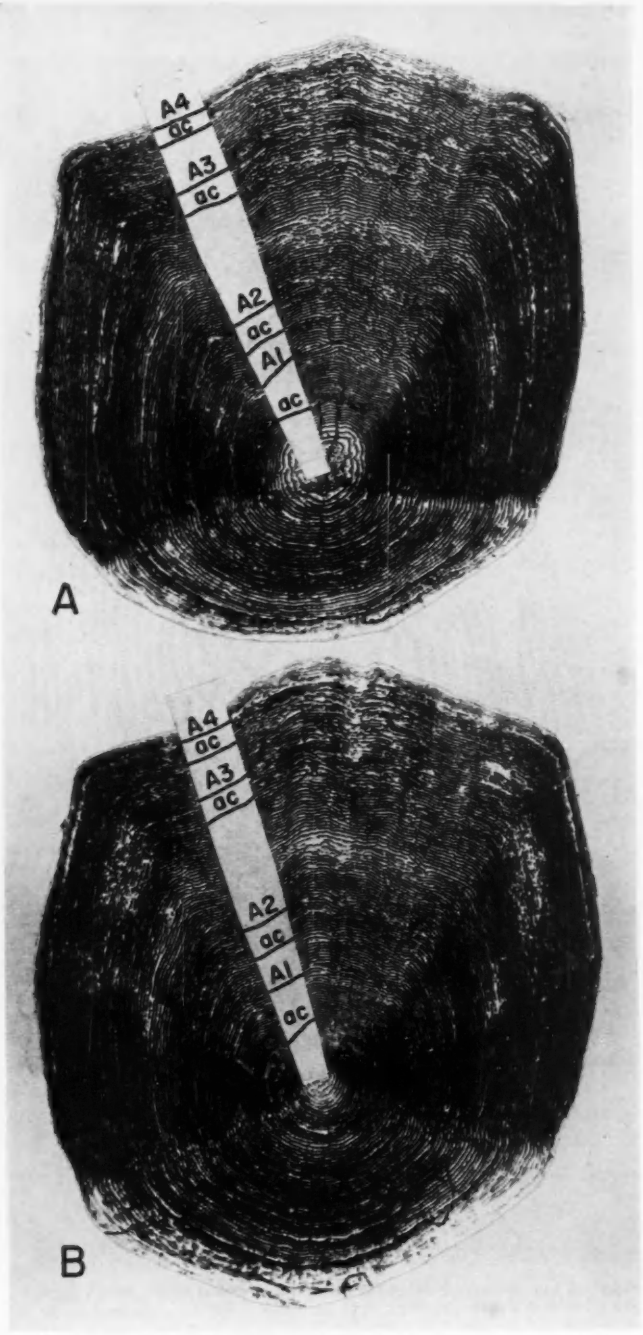

FIG. 1. A, Scale taken from a lake whitefish (specimen no. 5, Table I) on April 2, 1963, showing annulus (A4) starting to form; previous annuli and accessory checks (*ac*) are indicated; total length 399 mm. B, Scale from same fish taken April 26, 1963, showing fourth annulus completely formed; total length 404 mm.

FIG. 2. Scale taken from a lake whitefish (specimen no. 2, Table I) on September 17, 1962, before accessory check had formed; total length 114 mm.

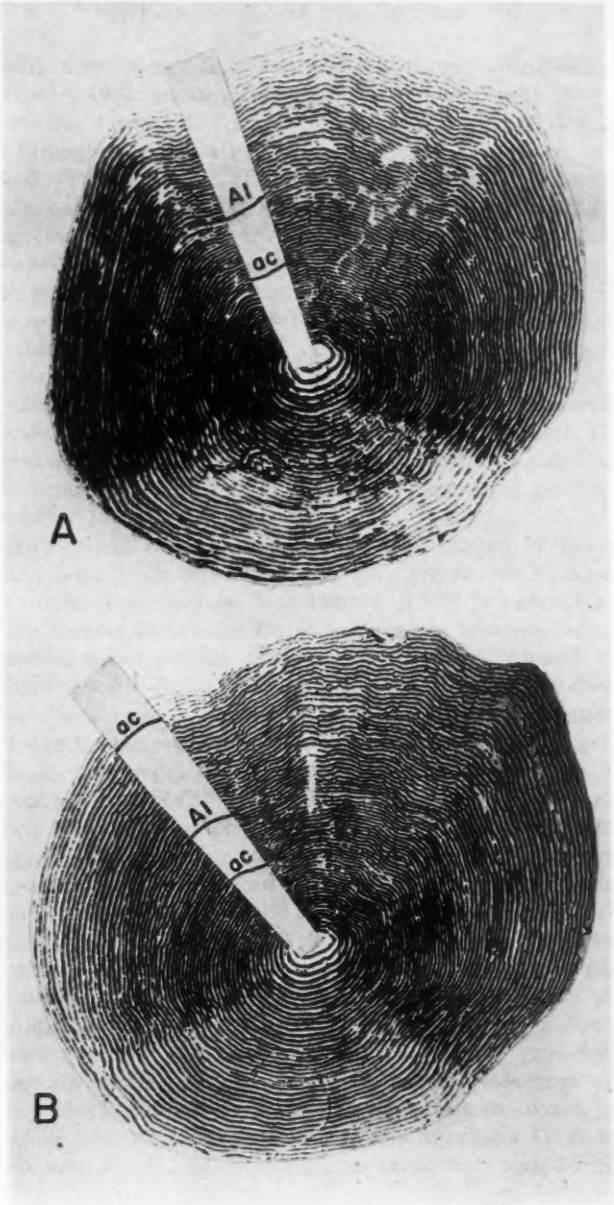

FIG. 3. A, Scale taken from a lake whitefish (specimen no. 2, Table I) on September 5, 1963, showing first accessory check and annulus; total length 246 mm. B, Scale from same fish taken on November 1, 1963, showing second accessory check completely formed; total length 264 mm.

The mark was absent (Fig. 2) on scales of three young-of-the-year whitefish in September 1962 but an accessory check had formed by early November. The first annulus formed in late March 1963. Scales from two of these fish that lived through the following fall showed only two strong marks (check and annulus) on September 5 (Fig. 3A) and October 7, but scales taken on November 4 had an additional strong check inside the margin (Fig. 3B).

The accessory check was not as well defined as the annulus but it could nearly always be detected. Comparison of calculated lengths from accessory checks with actual measurements in various months, verified the period when the checks were formed. Scales from unmarked fish that had died mid-November to January (all species and years) also had an accessory check just inside the margin. The widely spaced ridges between A2 and the next outer accessory check (*ac*) on Fig. 1 are commonly found in coregonid scales and reflect periods of fast growth (Van Oosten, 1923, 1929; Moffett, 1952). Seventeen sets of scales were taken from this fish (specimen 5, Table I) and they all showed the same characteristics and reflected very rapid growth during the early summer of 1961 at age II.

Accessory checks were not reported by Van Oosten (1923) on whitefish reared in the New York Aquarium, but they are known to occur for many lake populations of coregonids. Van Oosten (1929) illustrated a number of scales of lake herring from Lake Huron with marks between the annuli that I believe were accessory checks. Deason and Hile (1947) found that kiyis in Lake Michigan form definite annuli and prominent accessory checks on their scales despite small annual variation in water temperature (not over 2 or 3 degrees C) in the deep water where they live. Hile (1936) found that accessory checks occurred regularly on all scales of lake herring from Muskellunge Lake, Wisconsin, and Smith (1956) found false annuli on nearly all scales of Green Bay lake herring after the 2nd year of life. Barsukov (1960) described the annual formation of two rings on *Coregonus muksun*, a Russian whitefish.

Younger fish (ages 0–II) did not seem to form their accessory check earlier than older fish, although the check was more easily detected in young fish according to examinations of 10 marked fish and 34 unmarked fish. The clarity of the accessory check depended on the fish's growth rate after the check had formed. If the fish grew little after the late-October check formed on the scales, the check was faint and nearly indistinguishable on scales collected from November to January. If the fish did not grow after October, no scale material was deposited on the margin from November to March and an accessory check did not form. Of all the scales examined from marked fish, only three lake whitefish (larger than 400 mm, ages IV and V) and one bloater (241 mm, age II) failed to form an accessory check during 1 or more years.

GROWTH OF MARKED FISH

The marked fish grew fastest in the summer and slowest during the winter (Table II). The very largest fish in some groups showed no growth in length

TABLE II. Growth of various year-classes of marked fish in total length expressed as monthly percentage increases from annulus formation to annulus formation. Numbers of fish in parentheses; growth periods extend from the 16th day of one month to the 15th of the following month.

Period of growth	Lake whitefish			Bloater		Kiyi	Lake herring × bloater hybrid	Avg
	1958	1959	1962[a]	1960	1961	1961	1961	
1962–63								
Mar.–Apr.	6.1(2)	7.8(1)	–	4.6(1)	13.8(1)	9.5(2)	7.1(3)	8.2
Apr.–May	14.1(2)	8.7(1)	–	8.6(1)	14.2(1)	11.9(2)	8.4(3)	11.0
May–June	16.2(2)	11.0(1)	–	9.6(1)	10.8(1)	12.4(1)	12.1(2)	12.2
June–July	22.1(2)	11.0(1)	–	10.4(1)	9.7(1)	10.5(2)	14.6(3)	13.1
July–Aug.	21.2(2)	11.0(1)	–	18.2(1)	9.3(1)	9.5(2)	13.3(3)	13.8
Aug.–Sept.	14.2(2)	9.7(1)	–	15.4(1)	14.0(1)	9.3(2)	11.6(3)	12.4
Sept.–Oct.	6.1(2)	9.2(1)	4.6(3)	14.4(1)	14.7(1)	11.0(2)	8.0(3)	9.7
Oct.–Nov.	0.0(2)	6.6(1)	4.9(3)	8.8(1)	7.8(1)	6.0(2)	3.2(3)	5.4
Nov.–Dec.	0.0(2)	5.7(2)	4.9(3)	5.8(3)	2.9(1)	3.2(1)	4.2(3)	3.9
Dec.–Jan.	0.0(1)	6.6(1)	5.0(3)	1.9(1)	2.8(1)	4.8(2)	4.5(3)	3.7
Jan.–Feb.	0.0(1)	6.5(1)	3.2(3)	0.0(1)	0.0(1)	5.5(2)	5.2(3)	2.9
Feb.–Mar.	0.0(1)	6.2(2)	3.0(3)	2.3(1)	0.0(1)	6.4(2)	7.8(2)	3.7
1963–64								
Mar.–Apr.	–	–	4.9(3)	–	3.6(1)	–	10.5(2)	6.3
Apr.–May	–	–	7.2(5)	–	8.4(1)	–	11.2(2)	8.9
June–July	–	–	12.4(3)	–	18.9(1)	–	16.8(1)	16.0
July–Aug.	–	–	15.7(3)	–	15.7(1)	–	16.6(1)	16.0
Aug.–Sept.	–	–	14.0(3)	–	13.6(1)	–	11.6(1)	13.1
Sept.–Oct.	–	–	8.7(3)	–	8.6(1)	–	8.1(1)	8.5
Oct.–Nov.	–	–	8.5(3)	–	6.1(1)	–	3.6(1)	6.1
Nov.–Dec.	–	–	6.8(2)	–	1.9(1)	–	1.7(1)	3.5
Dec.–Jan.	–	–	5.3(2)	–	0.0(1)	–	1.8(1)	2.4
Jan.–Feb.	–	–	2.9[b]	–	2.7(1)	–	1.7(1)	2.4
Feb.–Mar.	–	–	3.7[b]	–	3.7(1)	–	3.7[b]	3.7

[a]Percentages for periods of growth in 1962–63 were obtained by dividing the amount of growth of each period by the growth to the first annulus.

[b]Data were not available for these months — the figures used are averages for corresponding periods of the 1962–63 growing season.

or on the scales during the winter. All others grew throughout the year; the slow winter growth did not cause cessation of scale growth, nor in most of the fish studied was the formation of accessory checks preceded or accompanied by a period of greatly reduced growth or no growth.

Data for whitefish used to illustrate growth covered the period September 1962 to December 1963 (Fig. 4). The annuli were formed at the start of a period of more rapid growth or during constant growth, and accessory checks were formed after the growth rate had become slower. The bloater, kiyi, and hybrid had growth curves similar to but somewhat more irregular than those of the whitefish. The irregularities for these species were random and could not be correlated with annuli or accessory marks on their scales.

As the size of the fish increased the rate of growth decreased, as is typical for whitefishes (Carlander, 1950). Growth of mature lake whitefish and bloaters was negligible over the winter. Two whitefish of the 1958 year-class (specimens no. 6 and 7) that matured in 1962 at age IV did not grow after late fall, and a whitefish of the 1959 year-class (specimen no. 5) that matured at age IV

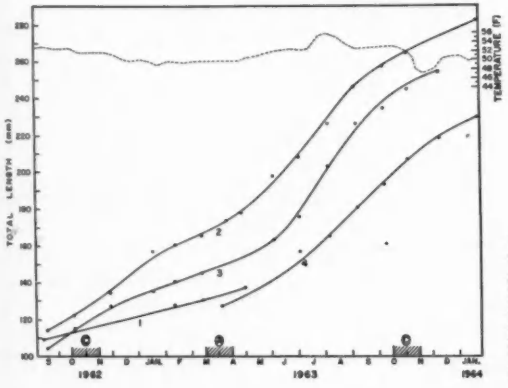

FIG. 4. Growth of four marked lake whitefish (1962 year-class) in 1962–64 and water temperature (broken line) in rearing tanks. Times of annulus formation (A) and accessory check formation (C) are indicated by circled letters. Numbers below curves are specimen numbers — see Table I.

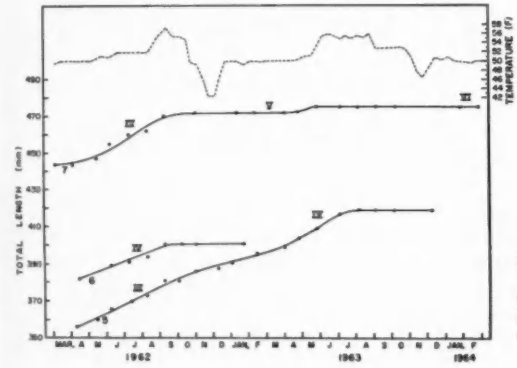

FIG. 5. Growth of lake whitefish (1958 and 1959 year-classes) in 1962–64 and water temperature (broken line in rearing tanks. Numbers below curves are specimen numbers — see Table I.

[b]Data were not available for these months — the figures used are averages for corresponding periods of the 1962–63 growing season.

in 1963 did not grow after August 1963 (Fig. 5). The latter fish was growing at a nearly constant rate in March–May 1963 while its fourth annulus (Fig. 1) was forming.

BODY-SCALE RELATIONSHIP

None of the coregonids exhibited a constant body-scale ratio. The body-scale relationship for all species was described by a straight line; intercepts on the axis of length ranged from 34 to 46 mm. The relations between magnified (×41) scale diameter (S) and total length (L) in millimeters of marked and unmarked coregonids, year-classes combined, were:

Species	Equation
Lake whitefish	$L = 34 + 1.057\ S$
Bloater	$L = 46 + 1.003\ S$
Lake herring × bloater hybrid	$L = 36 + 1.041\ S$
Kiyi	$L = 34 + 0.947\ S$

Lengths were calculated from the formula:

$$L_n = a + \frac{(L_t - a)}{S_t} S_n$$

where L_n equals length of fish at annulus (or accessory check) n, L_t equals fish length at time of scale removal, a equals the total length intercept of the body-scale equation, S_t equals the total scale diameter, and S_n equals the scale diameter at annulus (or accessory check) n. The calculated length of a particular marked fish at annulus n agreed very closely (±1–3 mm) with its measured length when annulus n was formed, or with its measured length in March–April before the series of scale collections was started.

Although the length intercepts for lake whitefish, bloaters, and kiyi were entirely satisfactory for calculation of growth in my experimental stocks, I do not suggest that they are generally applicable. The body-scale relation is known to vary from population to population of the same species. In Lake Superior, for example, the body-scale regression for lake whitefish has an intercept on the length axis in the Munising Bay population (Edsall, 1960) but passes through the origin in the Apostle Islands stocks (Dryer, 1963).

EFFECTS OF TEMPERATURE, FOOD, AND LIGHT ON FORMATION OF ANNULUS AND ACCESSORY CHECK

The constancy of water temperature at 50 F (10.0 C) during time of annulus formation (Fig. 4 and 6) proves that changing temperatures were not necessary to initiate formation of an annulus on the scales. Van Oosten (1923) suggested that adult fish (age VIII and IX) stopped growing in the winter and that rising water temperature (from 39 to 45 F; 3.9 to 7.2 C) in March and April caused annulus formation on whitefish reared in the New

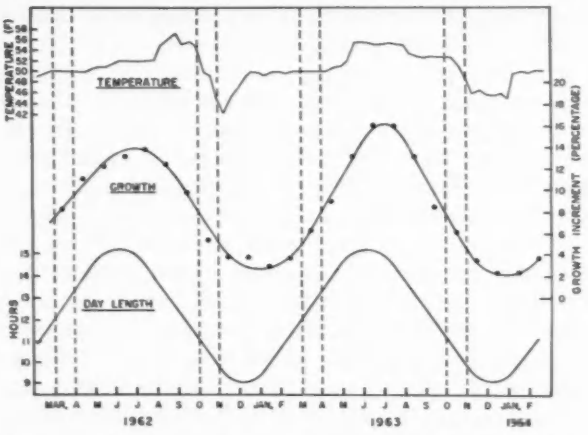

FIG. 6. Average monthly percentage increments of marked fish, hours of day length (sunrise to sunset), and average water temperatures, 1962–64. Periods of formation of annuli (March–April) and accessory checks (October–November) are indicated by vertical broken lines. Growth data from Table II.

York Aquarium. Only a few of the largest fish in the present study ceased growing during the winter.

During the period of accessory check formation, from mid-October to mid-November, water temperature decreased from 55.0 to 45.3 F (12.8–7.4 C) in 1962 and from 53.0 to 48.5 F (1.7–3.0 C) in 1963. The decrease during the same period was much less in 1958–61, however, and in some years it was less than 1 F (0.6 C): 53.6–51.2 F in 1958; 52.0–51.7 F in 1959; 52.8–52.0 F in 1960; and 51.9–49.4 F in 1961. Since all fish that were growing formed accessory checks in all years, it seems unlikely that temperature change alone may have caused formation of the accessory check.

In November 1962 and 1963 colder water from the holding pond was added to the water pumped into the tanks that held mature fish, to simulate water-temperature changes experienced by wild fish during the spawning period, but the changes (Fig. 5) came after the accessory checks had already formed. Cold water was not pumped into the tanks that held the immature fish of ages 0 and I (all species and year-classes). Consequently, they experienced less temperature variation in the fall (Fig. 4 for 1962); temperature did not fall below 49 F (9.4 C) for age 0, or 47 F (8.3 C) for age I. The use of spring water exclusively after the spawning period in mid-December kept the water temperature for all fish at 50 ± 0.3 F (10.0 ± 0.2 C) until late April or early May.

Neither the kind nor quantity of food was changed during the periods of formation of annuli and accessory checks, and no changes were observed in feeding during these periods. Mature whitefish ate less food during their spawning period, but spawning came after the check had formed. Mature

whitefish shorter than 400 mm resumed normal feeding after spawning; those longer than 400 mm did not feed as actively.

Prophylactic inoculations of chloroamphenicol and sulfa drugs, administered monthly, could not be correlated with the formation of marks on scales.

Seasonal change in growth rate of the marked coregonids was much more closely related to day length (hours from sunrise to sunset) than to changes in water temperature (Fig. 6) or any other known factor. Monthly increments were lowest in late December to early February, when periods of daylight were shortest. The annulus did not begin to form until mid-March, when periods of daylight had started to lengthen. Separate growth curves for the marked whitefish of the 1962 year-class (Fig. 4) show more clearly the actual growth of most fish studied. The fall accessory check on the marked fish formed during a period of decreasing growth but the decrease was not sudden, nor was it followed by rapid growth after check formation.

The day length that coincided with the periods of mark formation was 12.1 hr in the spring and 11.0 hr in the fall. The fall mark formed 1–2 months before the minimum day length of 9.1 hr in late December and the annulus formed near the middle of the period of rapid increase in day length (Fig. 6). No attempt was made to control seasonal variations in light, or light in the tank room. The internal illumination from electric lights did not dominate the natural light entering the large windows surrounding the tank room.

AN EXPLANATION OF FORMATION OF MARKS

The formation of a spring mark during a period of increasing growth or small change in growth, and a fall mark during a period of declining growth or little change in growth is contrary to the usual assumption that a mark forms at the onset of rapid growth after a period of reduced or no growth. It has also been widely assumed that temperature, through its effect on a fish's metabolic rate, is the principal factor of annulus formation (e.g., Graham, 1928; Beckman, 1943; Lagler et al., 1962).

An increase in fish length may determine the increase in the scale diameter but the pattern of circuli may be governed by additional factors. Wallin (1957) discussed the development of scale markings in considerable detail. He showed that the circuli are caused by calcification of ridges produced by pressure of the scale against the scale pocket. The periphery of the scale is proteinaceous and subject to formation of pressure ridges. As additional protein is deposited around the scale, the ridges are farther from the edge and become impregnated with inorganic salts. A typical single ridge (or circulus) begins to form and is calcified along the anterior region (imbedded part of the scale), and then continues to form and calcify posteriorly around the lateral edges of the scale until the ends meet along the posterior margin. As many as 3–10 circuli can be in various stages of completion at one time. If protein deposition stops or is reduced the incomplete ridges calcify to their tips. Keeton (1965) demonstrated that the proteinaceous margin (Wallin's ossoid growth zone) may not become reduced when the rate of protein deposition is temporarily retarded

or arrested, but the calcification of the ridges continues. If the proteinaceous growth resumes, the incomplete ridges do not resume their encircling growth, but a new ridge begins to form at the anterior edge, proceeds around the lateral edges, and eventually encircles the scale. This new ridge "cuts across" the incomplete circuli to complete the annulus characteristic of coregonids and many other fishes.

Possibly a physiological protein-demand cycle existed in the laboratory-reared coregonids that caused a periodic diversion of scale protein to support other metabolic functions. A biannual cycle would explain formation of annuli and accessory checks without a cessation in fish growth.

When coregonids become ripe in the fall, their gonad development may create a high protein demand. Many fish of the 1958 year-class that spawned in 1962 and 1963 showed evidence of scale resorption in late summer and fall, which suggests the diversion of scale protein. Immature fish did not show scale resorption in the fall but apparently experienced a sufficient change in their protein-deposition rate to form the characteristic accessory check.

The formation of an annulus in the spring may also reflect a metabolic change during the period that growth was increasing or nearly constant, but the reasons are unknown. Conceivably, the demand could be generated by increased activity during the time of rapidly lengthening days.

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REFERENCES

BARSUKOV, V. V. 1960. [Age of the Ob whitefish (*Coregonus muksun*) and some problems in theory.] Zool. Zh., **39**(10): 1525–1530. (Transl. from Russian IPST Cat. U.S. Dept. of Com., Washington, D.C., No. 908. p. 1–7.)

BECKMAN, W. C. 1943. Annulus formation on the scales of certain Michigan game fishes. Papers Mich. Acad. Sci., **28**: 281–312.

BROWN, M. E. 1946. The growth of two-year-old trout at a constant temperature. J. Exptl. Biol., **22**: 130–144.

CARLANDER, K. D. 1953. Handbook of freshwater fishery biology. Wm. C. Brown Co., Dubuque, Iowa. 429 p.

DEASON, H. J., AND R. HILE. 1947. Age and growth of the kiyi, *Leucichthys kiyi* Koelz, in Lake Michigan. Trans. Am. Fish. Soc., **74**(1944): 88–142.

DRYER, W. R. 1963. Age and growth of the whitefish in Lake Superior. U.S. Fish Wildlife Serv., Fish. Bull., **63**: 77–95.

EDSALL, T. A. 1960. The age and growth of the whitefish, *Coregonus clupeaformis*, of Munising Bay, Lake Superior. Trans. Am. Fish. Soc., **89**(4): 323–332.

GRAHAM, M. A. 1928. Studies of age determination in fish. Min. Agr. Fish., Fish. Invest. Ser. II, **11**(3): 50 p.

HILE, R. 1936. Age and growth of the cisco, *Leucichthys artedi* (LeSueur), in the lakes of the northeastern highlands, Wisconsin. U.S. Bur. Fish., Bull., **48**: 211–317.

HOGMAN, W. J. MS, 1965. The time of annulus formation and possible factors thereof, of six species of the genus *Coregonus* raised under artificial conditions. M.Sc. Thesis. School of Natural Resources, University of Michigan, Ann Arbor, Mich. 130 p.

KEETON, E. 1965. Application of Stoeltzner's method to determine growth of fish scales. Trans. Am. Fish. Soc., **94**(1): 93–94.

LAGLER, K. F., J. E. BARDACH, AND R. R. MILLER. 1962. Ichthyology. John Wiley and Sons, Inc., New York. xiii + 545 p.

MOFFETT, J. W. 1952. The study and interpretation of fish scales. Sci. Counselor, **15**(2): 40–42.

PARKER, R. R. 1963. Effects of formalin on length and weight of fishes. J. Fish. Res. Bd. Canada, **20**(6): 1441–1455.

SMITH, S. H. 1954. Method of producing plastic impressions of fish scales without using heat. Progressive Fish-Culturist, **16**(2): 75–78.

1956. Life history of lake herring of Green Bay, Lake Michigan. U.S. Fish Wildlife Serv., Fish. Bull., **57**: 87–135.

VAN OOSTEN, J. 1923. A study of the scales of whitefishes of known ages. Zoologica, **2**(17): 381–412.

1929. Life history of the lake herring (*Leucichthys artedi* LeSueur) of Lake Huron as revealed by its scales, with a critique of the scale method. Bull. U.S. Bur. Fish., **44**(1928): 265–428.

WALLIN, O. 1957. On the growth structure and developmental physiology of the scale of fishes. Rept. Inst. Freshwater Res., Drottningholm, **38**: 385–477.

Underwater Observations on Scallop (*Placopecten magellanicus*) Behaviour and Drag Efficiency[1]

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ABSTRACT

The efficiency of an 8-ft scallop drag was estimated from population density measurements by scuba divers. Density measurements had to be made with an enclosed quadrat because of scallop swimming activity. Scallops responded to approaching objects by facing away from them and swimming. A steep rise usually preceded level swimming at a mean height of 0.4 m from bottom. Point-to-point swimming distances of up to 4 m were recorded with ground speeds in excess of 67 cm/sec. Few scallops over 100 mm could be induced to swim.

Despite level bottom conditions, overall drag efficiency was low (2.1%) but increased progressively over the size range encountered (20–150 mm). Direct observations of drag function showed that swimming activity rather than selection by the drag was responsible for the low drag efficiency for the capture of scallops smaller than 100 mm.

An indirect fishing mortality was established for recessed scallops buried by the drag. Dragging resulted in dislodgement of dead shell to the substrate surface, and aggregation of benthic predators in the drag tracks.

INTRODUCTION

This paper reports on scuba observations carried out on the Richibucto scallop beds (Northumberland Strait, Gulf of St. Lawrence) August 7–19, 1967. Particular attention was paid to scallop behaviour in relation to fishing gear.

Scallop swimming has been reported frequently in the literature, especially relating to the European scallop, *Pecten maximus* (Lecomte, 1952; Baird, 1958; Hartnoll, 1967). Swimming activity has also been described in a number of other species, e.g. the Tasmanian scallop, *Notovola meridionalis* (Tate) (Olsen, 1955), and the bay scallop, *Aequipecten irradians* (Moore and Marshall, 1967).

No well-documented descriptions of *Placopecten magellanicus* swimming have been reported, although the spectacular locomotion of this species has been noted by several authors (Drew, 1906; Posgay, 1963) and is a subject of common knowledge among fishermen. Perhaps the importance of scallop swimming has been underestimated as a result of the tagging and recapture experiments of Baird (1954), Dickie (1955), and Posgay (1963) which formed the basis for previous estimates of drag efficiency. These authors found no evidence for extensive migrations in populations of adult scallops.

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Dickie (in Bourne, 1966) fished a gang of Digby drags with hoods of manilla twine attached above each drag. Large numbers of small scallops were caught in the hoods, which would normally have swum over the drag. This observation pointed to scallop swimming as a potential factor in reducing drag efficiency. The importance of this factor was established in the present study by direct observation of fishing gear in operation.

METHODS

The Richibucto scallop beds were chosen because of their shallow location with good underwater visibility. A small scallop fishery has operated in this area since 1951 (most recent report: Rowell and Lord, MS, 1965).

All operations were carried out from the M.B. *Pandalus* in 11 fath (~20 m) on smooth bottom, ranging in texture from mud to clean sand. At the time of observation the water column showed a sharp thermocline between 5 and 10 m of the surface; surface and bottom temperatures were around 18 and 5 C respectively. Tidal flow in the area is predominantly in a north–south direction. Bottom speeds of up to 0.7 knot[2] were recorded during the period of observation.

Exploratory tows with an 8-ft offshore scallop drag located scallop concentrations 3.9 nautical miles (7.2 km) north of the fairway buoy. A radar buoy was dropped and 10-min tows made out from the buoy. Catches averaged 50–75 animals per tow (8–9 kg, whole weight). Many specimens were below the commercial cull size (85–90 mm).

Drag Efficiency Estimate

Two methods were used to measure scallop density before dragging operations began. First, a 600-m length of 8-mm nylon lead-cored rope was laid on bottom with buoyed anchors at intervals along its length. The bottom rope was marked every 2 m with black tape. Two divers swam along the bottom rope with a 3-m cross stick and counted the scallops in successive $1\frac{1}{2} \times 2$-m rectangles on each side of the rope.

Population density was also measured, using a "corral" developed by D. J. Scarratt of this laboratory, for estimating the population density of lobsters. This consists of a square netting enclosure with sides 0.9 m high, enclosing an area of 21.2 m^2. The corral was lowered at regular intervals along two transects, parallel to the marker rope, and at approximately 50 m to either side of it. All scallops within the corral were brought to the surface in collecting bags. The interval between successive stations was judged by eye as approximately 70 m.

Three days later, the two end buoys of the 600-m rope were used as markers for the start and finish of a 600-m towing course with the offshore drag. Six tows were made parallel to the line and on each side of it, covering the

[2]1 knot = 1.853 km/hr.

stretches of bottom whose population density had been estimated previously by the corral quadrats.

Direct Drag Observations

Fishing operations were carried out with a modified offshore drag (Fig. 1), the forward drag bars being replaced by chains to reduce weight. Ropes were attached to the top of the pressure plate, so that a pair of divers could

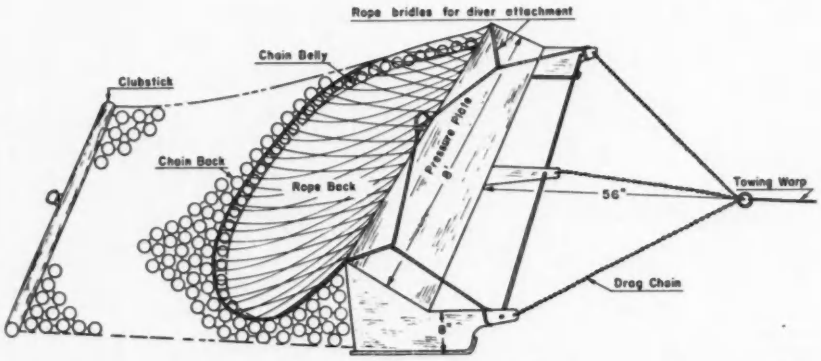

Fig. 1. Eight-foot offshore scallop drag modified to allow divers to accompany it in operation.

accompany the drag and observe its operation. After lowering the drag, divers swam down the cable and to signal readiness released a cloth bag containing air to the surface. Two accompanied drags were made, both of 5-min duration. On the first occasion the boat speed was below 2 knots. On the second occasion it was increased to approximately 2 knots after the first few minutes.

Scallop Orientation

Several dives were made to observe scallop orientations. Orientations were recorded on a check board (Fig. 2) constructed of 6-mm thick transparent plastic divided into octants, with attached underwater compass. Orientation of the shell hinge with respect to compass north was recorded for a random sample of approximately 50 scallops. A soft lead pencil was used to write on the roughened surface of the check board.

Behavioural observations were preceded and followed by an estimate of bottom current speed and direction using a simple current meter (Fig. 2). This vane meter (34 cm high; 46-cm base plate diam) consists of a direction vane, constructed of 0.5-mm galvanized sheeting which rotates freely on a 9-mm brass rod. The brass rod is continuous on the underside of the base plate as a spike which fixes the instrument vertically on bottom. The orientation of the direction vane is read off from the base plate after this has been adjusted to magnetic north by means of the underwater compass. A triangular vane

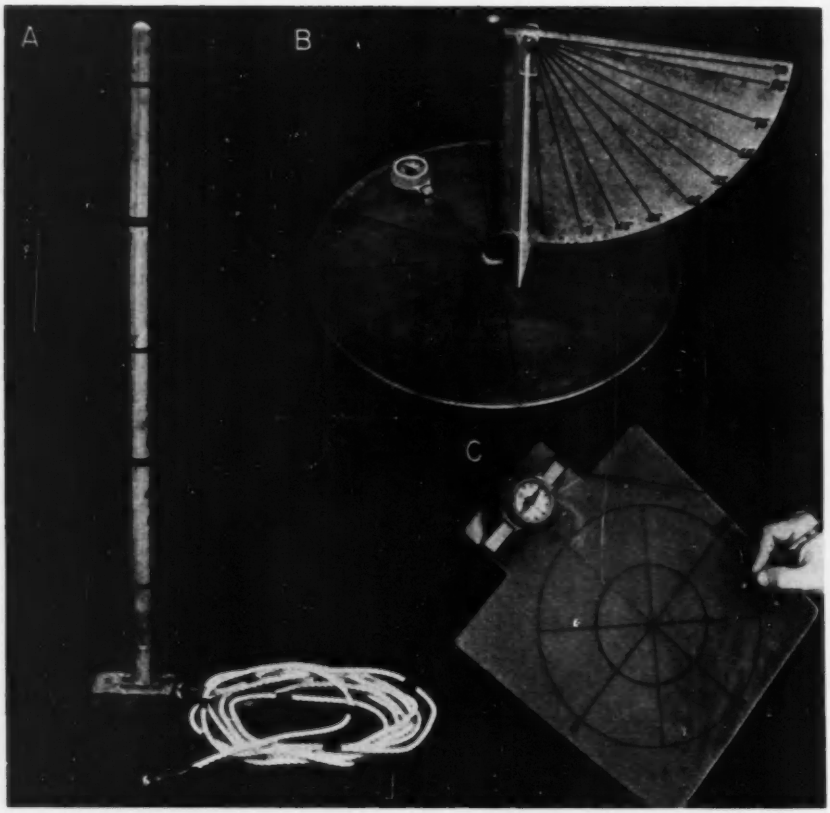

FIG. 2. (A) Equipment used to estimate point-to-point swimming distances and maximum heights off bottom reached by scallops during a flight response. (B) Vane meter measuring bottom current speed and direction. (C) Perspex check board with attached compass for recording scallop orientation and swimming direction.

of 3-mm white Perspex (10.3 cm across the base), pivoting on a brass screw passing horizontally through the direction vane, records current speed as degrees deflection from the vertical position. This current speed vane was later calibrated in a flow trough against the rate of revolution of an Ott meter propeller.

REACTION OF SCALLOPS TO DISTURBANCE

It was suspected from preliminary dives that scallops showed a directional response to the presence of a diver. However, a single observer who was close enough to bottom to induce a flight response was unable to determine swimming direction, since most disturbed scallops passed rapidly out of the field of view

of his mask. The following procedure was adopted to overcome this difficulty: one diver swam slowly along a line laid on bottom in a north–south direction, and attempted to touch any scallop in his path. The second diver swam above and slightly behind the first, noting the direction of movement of the scallop in relation to the first diver, and recording it on an orientation check board (Fig. 2).

On the first dive, only the initial swimming direction was recorded. On the second dive, the observer attempted to record the flight path of the scallop with respect to its initial position.

Current direction along the bottom was southerly (185°) during both series of observations. Two separate records were made on each dive: first, with the divers swimming with the current; second, with the divers swimming against the current.

Swimming Distance and Duration

The height above bottom attained by a swimming scallop was estimated using a wooden pole marked at 6-inch (15.2-cm) intervals and weighted at the lower end (Fig. 2). When placed on bottom, the pole was held upright on the weight by its own buoyancy. To the base of the pole was attached a measuring rope marked at 1-ft (0.31-m) intervals held by a second diver. The first diver dropped the weighted pole adjacent to a scallop, inducing a flight response, and used the calibrations on the pole to estimate maximum height reached above bottom. The second diver followed the scallop, counted the adductor contractions in flight, and on its termination collected the scallop and tightened the rope to estimate the distance travelled. While the rope was tight, the first diver determined the compass direction between initial and final positions. Both divers recorded results on a Perspex sheet, with attached scale for measuring the shell length of each scallop. Between 10 and 20 series of observations were carried out in a 15–20 min dive. The following day another series of dives were made in which an underwater watch was used to measure scallop swimming duration (seconds off bottom), and to relate it to the number of adductor contractions in flight.

DRAG EFFICIENCY AND REACTIONS OF SCALLOPS TO DRAG

Drag Efficiency

Population density estimates.— Scallops in the survey area were largely of one size: 55–75 mm long (Fig. 3), predominantly 5-ring animals. Approximately 90% of the population belonged to this age-group.

The first estimates of population density (August 8) were made by two divers along 22 m of the marker rope on clean sand (Table I). Close proximity to the diver induced a flight response in most scallops. Attempts to allow for this in estimating numbers of scallops in adjacent 3-m^2 areas led to counts by the first diver which were usually in excess of those recorded for adjacent areas by the second diver. For this reason, further use of the technique

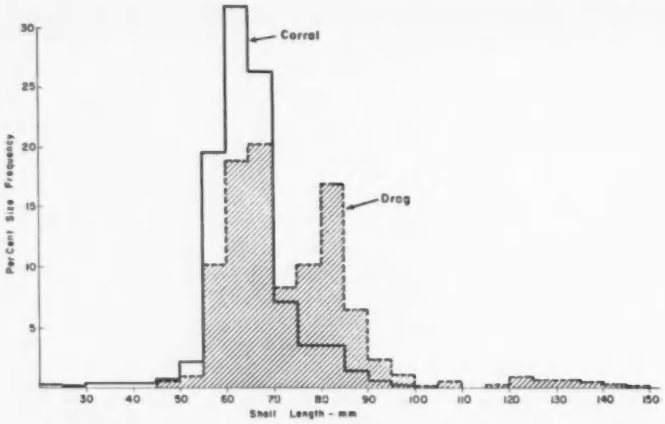

FIG. 3. Percent size frequencies of scallops collected by divers (corral estimates) (751 scallops) and those caught by dragging over the same area of bottom (685 scallops).

was discontinued. However, the mean for the two sets of observations gave an estimate for scallop density of 6.1/m².

The lobster corral provided a more reliable means of estimating population density, since it prevented scallops escaping from the enclosed area. Using this equipment, considerable variability was revealed both in bottom type and density of scallops in the area surveyed. Within a distance of 70 m there were rapid transitions from clean sand with a high density of scallops to mud bottom where they were less abundant.

Corral catches averaged 39.5 ± 26.1 (range 16–115), equivalent to a mean scallop density of 1.87/m². The estimated density of scallops on sand was 4.18/m². This second figure falls within the range of values obtained on sand using the rope transect method. Density on mud was 1.43/m².

Calculation of efficiency.— Scallop drag efficiency was calculated by comparing drag catches in 12 tows over a known distance of bottom (600 m), with direct measurements of scallop density made by corral counts in the same area. The average catch was 57.1 ± 38.2 scallops (range 13–136). Each

TABLE I. Numbers of scallops in adjacent 3-m² areas, estimated by paired divers.

												Total	Mean	SD
1st diver	15	24	–*	22	28	24	11	20	33	19	–*	196/9	21.78	6.59
2nd diver	9	10	15	25	20	19	19	12	17	10	15	171/11	15.54	5.03

*No estimate made.

tow covered an area of 1440.0 m² (width of the drag mouth: 2.4 m). An overall figure for drag efficiency of 2.1% was calculated.

Change in drag efficiency with size of scallop.— Drag efficiency was calculated separately for three different size-groups of scallops: (1) less than 50 mm; (2) 50–75 mm; (3) 75–100 mm. Progressively higher efficiencies of 0.69%, 1.39%, and 8.31% were calculated with increasing size (Table II).

TABLE II. Change in drag efficiency with size of scallop calculated from 19 corral estimates and 12 600-m tows with the offshore drag.

	Size-group (*mm*)				
	<50	50–75	75–100	>100	Total
Corral					
Total no. scallops collected in an area of 402.8 m²	17	662	71	0	750
Calculated no. scallops in area covered by one 600-m tow (1440 m²)	61.1	2376.9	254.7	–	2692.7
Drag					
Total no. scallops caught in 12 tows	5	395	254	31	685
Mean no./tow	0.42	32.92	21.17	2.58	57.09
Drag efficiency (%)	0.69	1.39	8.31	–	

The data for 100 mm+ scallops are inconclusive, since, although 31 were caught in the drag, none were taken in the corral. This size-group must therefore be present at a low density. If the efficiency estimate of 8.31% calculated for the 75–100-mm size-group is also true for the 100 mm+ scallops, then the 31 scallops captured by the drag represent a population of 373 scallops in the total area covered by the drag (17,280 m²). The expected number captured in the area covered by the corral samples (402.8 m²) would then be 8.7 scallops. The fact that no 100 mm+ scallops were captured in this area suggests that drag efficiency for this size-group is in excess of 8.31%.

OBSERVATIONS ON DRAG AND REACTIONS OF SCALLOPS

Direct observations were made on drag functioning and reactions of scallops to the drag by four divers. Their independent, written observations showed a high degree of correspondence, and are pooled in the following account.

Drag functioning.— At the beginning of the two, the tow chains and warp (Fig. 1) rose from the bottom at an angle of approximately 30° (with the towing shackle 2.3 ft (70 cm) off bottom) and the drag started forward smoothly. At no time was the gear observed to perform the "leaping" motions observed by Baird (1955) for European scallop gear which has teeth on the lower edge of the drag mouth.

At low towing speeds (below 2 knots) the leading edges of the chain back and belly were closely opposed, preventing material from entering the bag. Even at a towing speed of 2 knots the back and belly of the drag had only separated about 6 inches (15 cm) in the centre of the mouth of the bag. Accumulated shell debris and rocks still blocked the entrance, contributing to the "bulldozing" effect reported by Cameron (MS, 1955).

Baird (1966) noted that the rope back of the European scallop drag provided a selection mechanism for eliminating smaller scallops. This was also true for the Canadian offshore drag. Below 2 knots the rope back was under low tension (Fig. 4A) with the meshes wide open, allowing many scallops to swim through freely. With an increase in speed or greater weight of material in the bag, the rope back came under tension (Fig. 4B, 5A) and a reduction in the horizontal distance between adjacent meshes was noted. As a result, fewer swimming scallops were able to escape by this route.

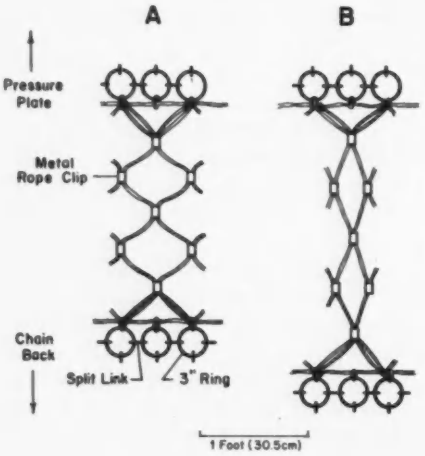

FIG. 4. Effect of tension on the dimensions of the rope back. (A) Low towing speed, drag empty; (B) high towing speed, drag full.

Reactions of scallops to drag.— While swimming down the towing warp to the stationary drag, divers noted that vibrations of the warp lying on bottom caused scallops to swim out of the immediate path of the drag. This effect would not, of course, be appreciable in normal fishing operations where dragging commences almost immediately the gear reaches bottom.

At low speeds (below 2 knots) many scallops rose from bottom just below where the warp met the drag chains, a semi-circular disturbance being noted at the "V" of the warp, approximately 4 ft (1.2 m) in front of the leading edge of the drag. All disturbed scallops showed a resultant direction of motion away from the drag. Many escaped to the side of the tow path. Animals of 70-mm shell length and over maintained the speed of the drag at low towing speeds; smaller scallops fell behind the drag while swimming. Of those animals swimming in the path of the drag, some fell in front of the drag at the end of

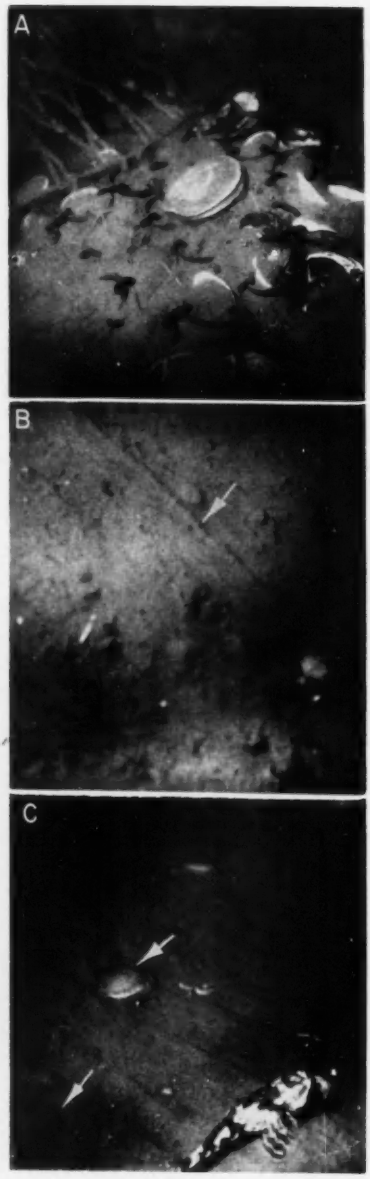

FIG. 5. (A) Drag stationary on bottom after fishing, showing clogging of chain back by sediment and shell debris, and meshes of the rope back. (B) Drag tracks on mud bottom. A recently immigrant scallop marks one end of a furrow produced by the lateral skids of the drag (arrow). (C) Mud bottom showing series of ridges in the centre of drag track. Two scallops (arrows) have been forced into the substrate by the passage of the drag.

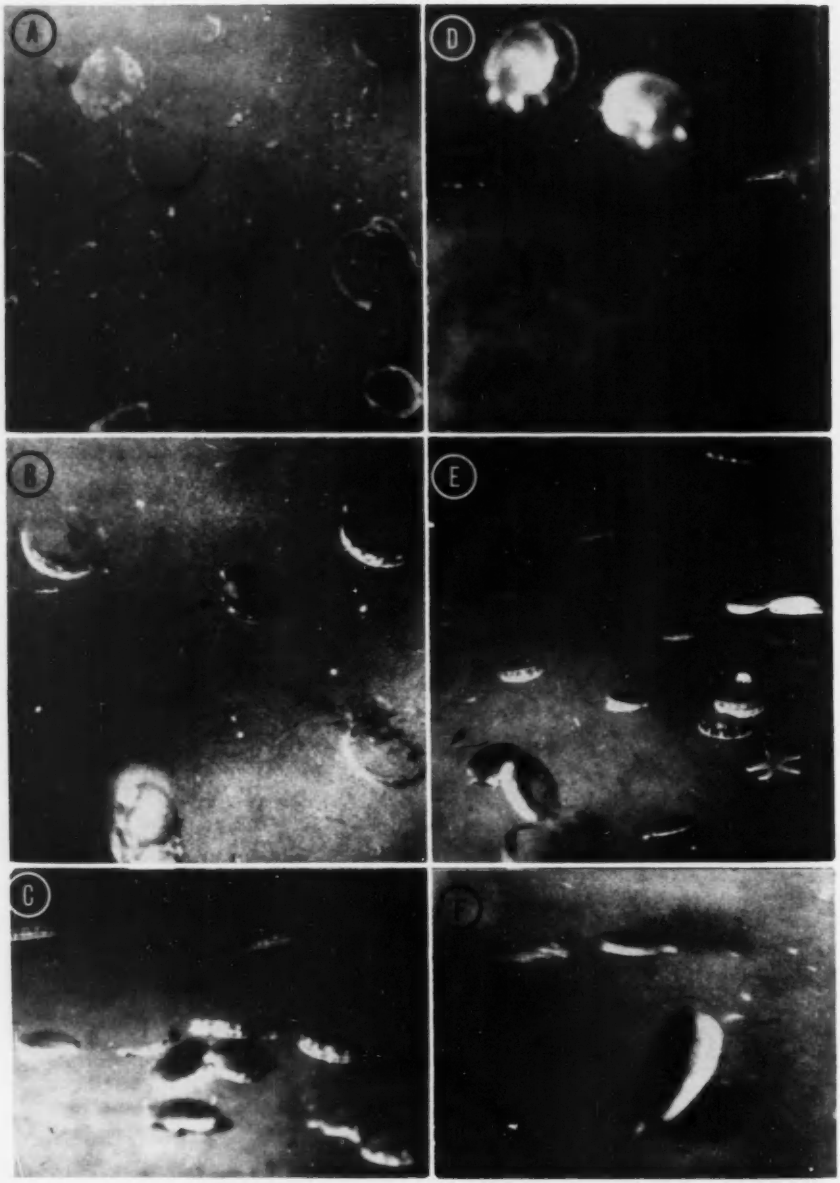

FIG. 6. (A) Debris dislodged from the substrate by the drag. (B) Recently motile scallop (arrow) contrasts with nonmotile specimens having sediment on the upper shell. (C) High density area on sand. Undulations caused by recessing activity evident from varied attitudes of scallops on bottom. (D–F) Stages in the flight response: (D) initial steep rise from bottom; (E) level flight; (F) passive sinking.

a flight response, either to pass under the pressure plate or to take off once more. At higher speeds (around 2 knots) the drag passed underneath many swimming scallops which escaped by dropping behind the pressure plate when they ceased swimming. At this speed most scallops took off about 3 ft (0.9 m) in front of the drag. Some were picked up as they rose from bottom. Many still escaped, since they rose quickly above the height of the pressure plate, 9 inches (23 cm) off bottom. These often impeded observation by striking the mask and mouthpiece of the observer. Fewer individuals escaped to the side of the drag. Several specimens were held by water pressure on the leading edge of the towing bar.

Effects of dragging.— On conclusion of each tow, the two divers riding the drag made immediate observations on the area of bottom fished over by the gear. Large numbers of scallops were found in the track of the drag. Approximately 30% of these were recessed animals which had been partly buried by the drag.

Approximately half the scallops in the drag track had upper shell valves free of sediment (Fig. 6B), and must have entered the drag track after the sediment stirred up behind the drag had subsided.

Drag tracks showed up prominently on bottom; two parallel furrows approximately 3 cm deep were evidently made by the lateral skids (Fig. 5B); a series of smooth ridges between them (Fig. 5C) were probably caused by the individual rows of rings in the drag belly. Dislodged pieces of dead shell were more evident within the tracks than on the surrounding bottom (Fig. 6A).

Passage of the drag evidently attracted numerous benthic predators: one large *Asterias vulgaris* was observed eating a 65-mm scallop in the drag track half an hour after fishing. Many sculpin and flatfish were also observed foraging in the track of the drag.

OTHER SCALLOP BEHAVIOUR

Recessing and Byssus Attachment

The undulating and pitted appearance of the sand bottom in areas of high scallop density (Fig. 6) suggested that this effect was a consequence of scallop activity. This hypothesis was supported by the high proportion of scallops occupying recessed areas in the bottom. Separate collections of recessed and unrecessed scallops revealed that recessed scallops were predominantly larger than average (Fig. 7). Recessed scallops were easily collected since they showed no escape reaction when approached by a diver. The shell valves of recessed individuals were closely opposed, with the pallial tentacles withdrawn. Erosion of surface sand in the depressions revealed an underlying stratum of dead shell just below the soil surface. An examination of recessed specimens on deck revealed that about 40% were attached by byssus threads to shell fragments from the bottom of the recessed area.

Recessing appears to be a consequence of the rejection response, a violent clapping of the shell valves which causes a cloud of sand to be blown away

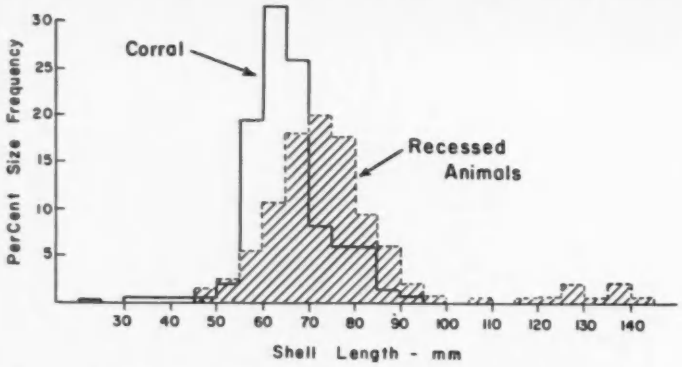

FIG. 7. Percent size frequency of recessed animals compared with size frequency of corral collections.

from the margins of the shell. Sediment deflected in this way from the sides of the pit is deposited on the upper shell valve, so that undisturbed scallops are invariably covered with a fine layer of sand. This sediment layer is shed in the first stage of swimming, so that recently moved individuals have the upper shell valve free of sediment (Fig. 6B).

ORIENTATION

Two successive dives were made on August 11 to determine scallop orientation with respect to compass north. On both occasions, the direction of the bottom current was 210° (magnetic), falling off in speed during the period of observation from above 0.30 to 0.24 m/sec. The scallop population exhibited a definite orientation on both occasions which appeared to differ from random (Fig. 8). On the first dive (0930 hr) the mean direction of orientation of the pallial gape was 78° (magnetic), most scallops facing obliquely into the current; on the second dive over the same area (1000 hr), the mean direction of orientation was 26° (magnetic) roughly northeast, most scallops facing more closely into the current.

SWIMMING

Most small scallops swam at a distance of 0.5–3.0 ft (15–91 cm) from an approaching object. Scallops over 100 mm long were generally recessed, and only swam in response to tactile stimulation, if at all.

Observations on the response of unrecessed scallops to an approaching diver revealed that the resultant swimming direction was invariably away from a disturbance (Fig. 9), when approached either downstream or upstream to the direction of tidal flow. When moving over a densely populated bottom, the diver was always the centre of a semi-circular front of dispersing scallops.

Close observation revealed that if approached from the hinge direction, a scallop would swim without preliminary reorientation. However, if ap-

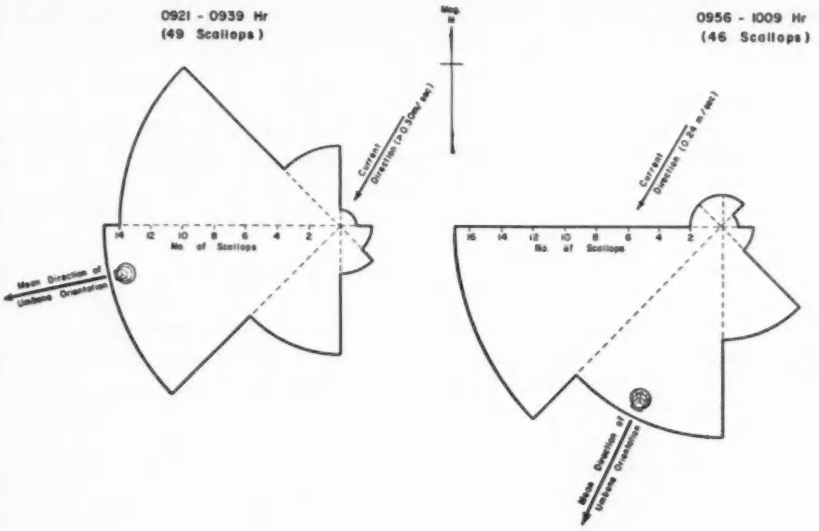

FIG. 8. Orientations of shell umbones with respect to magnetic north recorded during two successive dives on the same population of scallops (August 11, 1967). (The orientation of the shell umbone is at 180° to the midpoint of the pallial gape.)

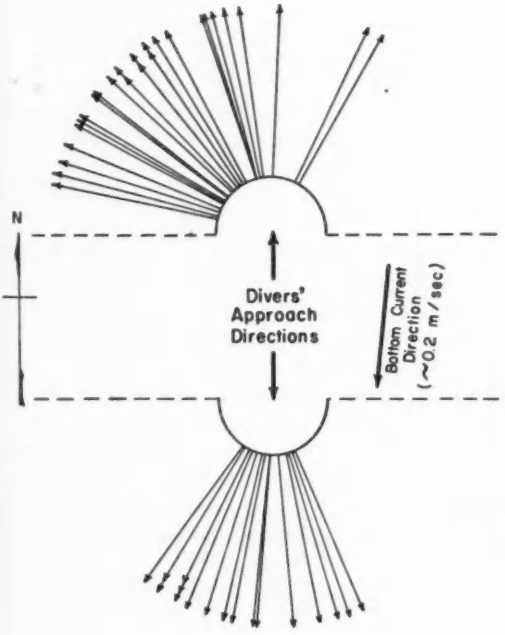

FIG. 9. Initial swimming directions of scallops relative to the approach direction of a disturbance. Divers swam first south, then north (with and against the current) along a line laid on bottom. (One arrow head = one scallop.)

proached from the direction of the pallial gape, the scallop rotated rapidly to face away from the disturbance before swimming. In consequence, the initial swimming direction was invariably away from a disturbance, irrespective of its approach direction relative to the axis of the shell.

Because the flight response was seen from its origin, deviations from straight-line swimming were inevitably exaggerated; however, attempts to trace the direction of locomotion showed that scallops often veered off to either side of their initial swimming direction after travelling a short distance (Fig. 10).

Three phases were distinguished in a typical instance of scallop swimming (Fig. 6D–F, 11):

(1) A rapid ascent from bottom at an angle of 30–50°. This stage occupies several seconds (the first 5 or 6 adductor contractions) and usually carries the scallop between 1 and 2 ft (30–60 cm) off bottom.

(2) A levelling off and completion of active swimming. The scallop either maintains a constant height, or rises slightly.

(3) A passive descent to bottom at the end of active swimming. This occurs in a series of side slips, the scallop invariably reaching bottom with the convex shell valve uppermost.

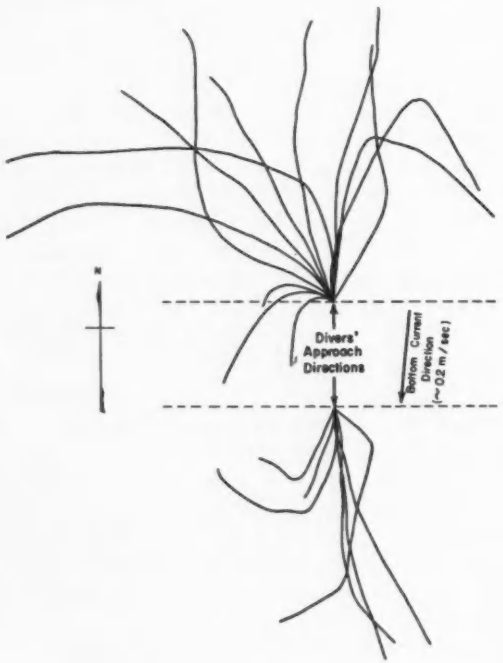

FIG. 10. Swimming directions of scallops moving in response to divers, estimated from the point of flight initiation. (Procedure as for Fig. 9: one tracing for each scallop.)

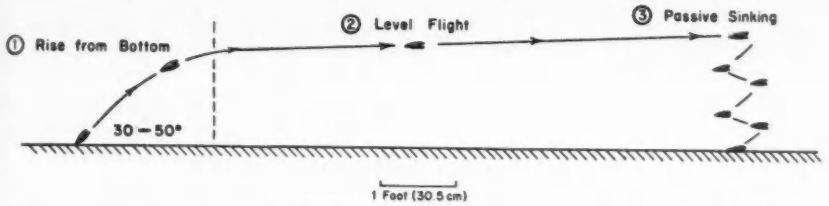

FIG. 11. Typical configuration of swimming path during *P. magellanicus* flight response.

Measurements of distance and duration of swimming were made on separate occasions. The straight-line distance between initial and final positions (point-to-point swimming distance) was significantly correlated both with the maximum height reached off bottom (Table III) and with the number

TABLE III. Point-to-point swimming distance (d) and height off bottom (h) during flight response ($h = 0.373 + 0.142\ d$). Correlation coefficient = 0.706 (significant at 1% level).

Maximum height off bottom	Point-to-point swimming distance (*ft*)[a]											
(*ft*)	3	4	5	6	7	8	9	10	11	12	13	14
3.0							1					
2.5												1
2.0				1		1	1	1	1			
1.5			1		1	2	1	1	1		1	
1.0	2	2	2	2		1						
0.5	2		2									

[a]1 ft = 0.305 m.

of adductor contractions in the active swimming phase (Table IV). A significant correlation was also found between the number of adductor contractions and the duration of flight response (seconds off bottom) (Table V). Scallops covered a mean horizontal distance of 7.5 ft (2.3 m), with a mean expenditure of 12 adductor contractions, and reached a height of 1.4 ft (0.4 m) off bottom. Few scallops larger than 100 mm could be induced to swim. These showed a greatly reduced locomotor ability, covering a horizontal distance of approximately 3 ft (0.9 m) with 6–8 adductor contractions, rarely rising more than 3–4 inches (7.5–10 cm) off bottom.

No evidence was found that swimming distance was related to bottom current direction, as noted for *Aequipecten irradians* by Moore and Marshall (1967).

Swimming speed.— It proved impossible to make simultaneous measurements of swimming distance and duration, so that a direct calculation

TABLE IV. Point-to-point swimming distance (d) and number of adductor contractions (c) during flight response ($c = 9.050 + 0.407\ d$). Correlation coefficient = 0.391 (significant at 5% level).

No. of adductor contractions in flight	Point-to-point swimming distance (*ft*)[a]											
	3	4	5	6	7	8	9	10	11	12	13	14
18		1		1								
17			1						1			
16								1				
15												
14							2	1				1
13						1	1				1	
12					1							
11	1			1		1						
10	1		2			1			1			
9			1			1						
8	2	1	1	1								
7												

[a]1 ft = 0.305 m.

TABLE V. Number of adductor contractions (c) and time off bottom (t) in flight response ($c = 5.237 + 0.904\ t$). Correlation coefficient = 0.638 (significant at 1% level).

No. of adductor contractions in flight	Time off bottom (*sec*)								
	5	6	7	8	9	10	11	12	13
21								1	1
20									
19					1	1			
18									
17									1
16					1		2		
15				2	1				1
14									
13			2		2	1		1	
12			1		1	1			
11			2	1				1	1
10		1	1						
9		2							
8	2								

of swimming speed cannot be made from the data in Tables IV and V. However, the number of adductor contractions in flight (c) was related both to the point-to-point swimming distance (d) and the time off bottom (t). Assuming a linear relationship exists in both cases, the method of least squares gives us the following equations:

$$c = 5.237 + 0.904t \quad \ldots\ldots(1)$$

$$c = 9.050 + 0.407d \quad \ldots\ldots(2).$$

Thus:

$$d = -9.369 + 2.222t \quad \ldots\ldots(3).$$

This gives us an estimate for the mean point-to-point swimming speed of 2.22 ft/sec (67.7 cm/sec), approximately 1.5 mph. This is an underestimate of actual swimming speed because the distance measured point to point is always less than the actual distance travelled. It does not take into account horizontal deviations from straight-line flight (Fig. 10) nor variations in height off bottom, especially during the early flight period. Also, time off bottom (t) includes both active swimming time and the time for passive descent to bottom after the adductor contractions have ceased. Despite these reservations, the estimated swimming speed is of the same order as that observed by divers on the scallop dredge; scallops were observed to maintain the speed of the drag at between 1 and 2 knots.

DISCUSSION

Scallop Orientation and Swimming

Direct observations by divers support the hypothesis that scallop swimming is principally an avoidance reaction. No evidence was obtained for movements in an undisturbed population. Although swimming ability undoubtedly evolved as a method of avoiding predators, both divers and scallop drags elicit a flight response in scallops below 100 mm shell length. The rapid rise from bottom, and laterally curved flight path allow a swimming scallop to rise above the mouth of the drag and swim out of its immediate path.

Under undisturbed conditions, the population showed a preferred direction of orientation. Whether this was related to the direction of tidal flow, as has been suggested by Hartnoll (1967) for *Pecten*, has yet to be confirmed for *Placopecten*. Some evidence was found that the overall direction of orientation changes over short periods of time.

Swimming direction is independent of the preferred direction of orientation of the undisturbed population. Scallops face away from a disturbance before swimming.

Circumstantial evidence suggests that detection of an approaching stimulus is visual:

(1) An avoidance reaction was only shown to an approaching stimulus by individuals with widely separated shell valves which prominently displayed the rows of mantle eyes.

(2) Recessed individuals were unresponsive to approach. The shell valves of these individuals were adducted, covering the mantle edge.

(3) Scallops could detect an object moving slowly towards them at a distance of 15–91 cm, even if care was taken to avoid water disturbance.

(4) The water clarity and light level during the period of observation might perhaps favour a visual response.

Scallop Drag Efficiency

Interpretation of the experimental dragging results in the light of direct observations of drag operation suggested the following conclusions:

(1) The offshore drag is most efficient for the capture of large scallops. Size selection by the drag invalidates conclusions drawn from drag samples as to the size composition or number of year-classes in the benthic population. Drag selection caused a unimodal population to show two modes in the range 50–100 mm (Fig. 3).

(2) The low drag efficiency ($<8.3\%$) for the capture of scallops below 100 mm shell length was observed to be mainly due to the ability of this size-group to elude the drag by swimming. The higher drag efficiency ($>8.3\%$) for larger scallops appears to be mainly due to their lack of motility. Some loss of drag efficiency for the capture of larger scallops must be due to the habit of recessing in sand.

(3) Conclusions drawn from dragging as to population density are of little value even with accurate figures for drag efficiency. Drag selection depends to a great extent on bottom type, which is highly variable on different scallop grounds.

Direct observation of the offshore drag in operation revealed a number of ways in which scallops can escape the drag, in addition to the ring selection effect investigated by earlier workers (Dickie, 1955; Medcof, 1952; Bourne, 1965). These may be summarized as follows:

Scallops in the path of the drag may elude its action by (1) swimming over the top of the drag; (2) swimming laterally out of the drag path.

Scallops passing under the pressure plate may still escape by (3) swimming through the rope back; (4) passing under the drag chain. Mechanisms 1–3 were only observed in smaller, more active scallops (<90 mm: the approximate commercial cull size). Mechanism 4 applies particularly to large, recessed scallops which are generally inactive.

Further investigation will be needed to establish the effect of swimming on gear selection at the fishing speeds used in commercial operations (3–4½ knots: Bourne, 1964). The ability to escape over the drag evidently depends on drag speed, scallop reaction distance, and the time taken to reach the height of the drag mouth (23 cm). If we assume that the calculated ground speed of 67.7 cm/sec applies to the active phase of the flight response, then a scallop rising away from a drag at 45° to the bottom will reach a height of 23 cm in 0.34 sec. A drag travelling at 4½ knots (2.32 m/sec) should then capture all scallops reacting less than 0.56 m from the front edge of the pressure plate.

This is less than the observed reaction distance (0.9 m) at 2 knots, and argues for the possible application of mechanism 1 (above) at even higher speeds than those directly observed.

Several improvements to the offshore drag can be envisaged which would lead to greater sampling efficiency for smaller scallops:

(*a*) To eliminate mechanism 1, the upper leading edge of the drag should be raised approximately 1 m off bottom.

(*b*) Mechanism 2 could be reduced by some sort of herding effect, similar to that which has been established for groundfish capture by the otter trawl. With present gear design, the point of convergence of the drag bars rather than the leading edge of the drag appears to provide the stimulus for swimming. This is likely to favour dispersal to the sides of the drag path. This effect should be taken into account when calculating optimum fishing speeds for the capture of swimming scallops. The greater height of the head rope (1–2 m) and herding effect of the wings in the scallop trawl are probably responsible for its high efficiency (Bourne, 1966) for the capture of small scallops.

(*c*) A reduction of rope back mesh size would reduce escapement of scallops by this route. Baird (1959) showed that size selection with the European scallop drag occurs largely through the back of the bag.

All of these considerations only apply where it is desirable to capture scallops efficiently as soon as they are recruited to a fishery, or for research purposes of sampling the pre-recruit year-classes. The first provision might apply when early recruits are present in great abundance, and it is desirable to harvest them rapidly to avoid mass mortality occurring (Gulf of St. Lawrence fishery — Dickie and Medcof (1963)). However, considerations of size selection have usually been directed to reducing capture of "discards" (Medcof, 1952; Medcof and Bourne, 1964; Bourne, 1965). In this case, the above criteria will be ones to avoid in drag design. In fact, the present design of the offshore drag shows its highest efficiency for larger, nonmotile scallops. In either case a general improvement of drag performance might result from a greater separation of the back and belly of the drag.

Effects of Dragging

The scallop drag has three important effects in addition to the reduction of population by capture:

(*a*) *Indirect mortality.*— Work by Medcof and Bourne (1964) has already established that damage to discards on deck is an important cause of fishing mortality. Direct observation confirmed another type of mortality described by these authors — damage to uncaptured scallops on the bottom. Shell damage can result from scallops passing under the drag. These animals may be forced into the bottom and the mantle cavity packed with sediment. This damages the soft tissues, especially of the mantle edge. Specimens from silty areas often show "mud blisters" under the mantle as a result of soil being forced between the mantle edge and the shell.

Aggregation of fish and predatory invertebrates in the drag track immediately after fishing may also lead to predation on scallops temporarily immobilized by drag damage.

(*b*) *Disturbance of the bottom.*— The action of a light (800-lb[3]) 8-ft scallop drag had a noticeable effect on bottom appearance in the study area. The most obvious effect was the dislodging of dead shell to the soil surface. This effect is likely to be more pronounced with the commercial (12-ft) scallop drag which weighs between 1300–1500 lb when empty and upwards of 8000 lb when full of rocks (Bourne, 1964).

(*c*) *Dispersion and harvesting efficiency.*— The efficiency of the offshore drag in initiating swimming in small scallops was established by direct observation. This was true not only for scallops in the direct path of the drag, but for animals to either side, which swam into the track after passage of the drag. One likely result of this dispersion is that population density will remain relatively constant over an area being fished, as a result of dispersion of scallops from high density patches into adjacent areas where the population has been reduced by dragging. As a result, scallop density is likely to fall quickly below a commercially profitable level with a relatively low depletion of stocks.

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REFERENCES

Baird, F. T. 1954. Migration of the deep sea scallop (*Pecten magellanicus*). Maine Dept. Sea Shore Fish., Fish. Circ. No. 14. 8 p.

Baird, R. H. 1955. A preliminary report on a new type of commercial escallop dredge. J. Conseil, Conseil Perm. Intern. Exploration Mer, **20**(3): 290–294.

1958. On the swimming behaviour of escallops (*Pecten maximus* L.). Proc. Maloc. Soc., London, **33**: 67–71.

1959. Factors affecting the efficiency of dredges. *In* Helmar Kristjonsson [ed.] Modern fishing gear of the world. Fishing News (Books) Ltd., London, Eng. p. 222–224.

1966. Notes on an escallop (*Pecten maximus*) population in Hollyhead Harbour. J. Marine Biol. Assoc. U.K., **46**: 33–47.

Bourne, N. 1964. Scallops and the offshore fishery of the Maritimes. Bull. Fish. Res. Bd. Canada, No. 145. 60 p.

1965. A comparison of catches by 3- and 4-inch rings on offshore scallop drags. J. Fish. Res. Bd. Canada, **22**(2): 313–333.

1966. Relative fishing efficiency and selection of three types of scallop drags. Intern. Comm. Northwest Atlantic Fish., Res. Bull. No. 3. p. 15–25.

[3]1 lb = 0.454 kg.

CAMERON, W. M. MS, 1955. An investigation of scallop-drag operation with underwater television equipment. Natl. Res. Council Canada, Radio & Electrical Eng. Div. (Pam. ERB-378/6). 7 p.

DICKIE, L. M. 1955. Fluctuations in abundance of the giant scallop, *Placopecten magellanicus* (Gmelin), in the Digby area of the Bay of Fundy. J. Fish. Res. Bd. Canada, **12**(6): 797–857.

DICKIE, L. M., AND J. C. MEDCOF. 1963. Causes of mass mortalities of scallops (*Placopecten magellanicus*) in the southwestern Gulf of St. Lawrence. Ibid., **20**(2): 451–482.

DREW, G. A. 1906. The habits, anatomy, and embryology of the giant scallop (*Pecten tenuicostatus*, Mighels). The Univ. Maine Studies, No. 6. 71 p. + 17 pls.

HARTNOLL, R. G. 1967. An investigation of the movement of the scallop, *Pecten maximus*. Helgolaender Wiss. Meeresuntersuch., **15**(1–4): 523–533.

LECOMTE, J. 1952. Réactions de fuite des pectens en présence des astérides. Vie et Milieu, **3**(1): 57–60.

MEDCOF, J. C. 1952. Modification of drags to protect small scallops. Fish. Res. Bd. Canada, Atlantic Progr. Rept. No. 52. p. 9–14.

MEDCOF, J. C., AND N. BOURNE. 1964. Causes of mortality of the sea scallop, *Placopecten magellanicus*. Proc. Natl. Shellfish. Assoc., **53**(1962): 33–50.

MOORE, J. K., AND N. MARSHALL. 1967. An analysis of the movements of the bay scallop, *Aequipecten irradians*, in a shallow estuary. Ibid., **57**(1966): 77–82.

OLSEN, A. M. 1955. Underwater studies on the Tasmanian commercial scallop, *Notovola meridionalis* (Tate) (Lamellibranchiata: Pectinidae). Australian J. Marine Freshwater Res., **6**(3): 392–409.

POSGAY, J. A. 1963. Tagging as a technique in population studies of the sea scallop. North Atlantic Fish Marking Symp., 1961. Intern. Comm. Northwest Atlantic Fish., Spec. Publ. No. 4. p. 268–271.

ROWELL, T. W., AND E. I. LORD. MS, 1965. Gulf of St. Lawrence scallop survey — 1965. Fish. Res. Bd. Canada, MS Rept. Ser. (Biol.), No. 842. 38 p.

Laboratory Experiments on the Orientation of the Adult American Eel, *Anguilla rostrata*[1,2]

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ABSTRACT

Adult American eels, *Anguilla rostrata*, caught on their spawning migration downriver, were filmed while in a circular tank, and the orientation of the eels was analysed by determining the direction of alignment of each eel in each frame of the time-lapse film. When allowed a view only of the sky, the eels oriented southward, in the direction of their supposed breeding area in the region of the Sargasso Sea. This southward orientation continued even if the eels were denied a view of the sky, provided they were subject to the diel rhythm of light–dark. Thus the eel seems capable of noncelestial orientation; the physical basis of this is not known.

INTRODUCTION

The American eel (Apodes *Anguilla rostrata* LeSueur) is a catadromous fish; after spending up to 10 years in lakes, rivers, estuaries, and sublittoral waters, eels migrate in the fall to the vicinity of the Sargasso Sea to spawn (Schmidt, 1924; Bertin, 1956). However, Vladykov (1964) considered that the data compiled by Schmidt were incomplete, and that the distribution of the adult American eel suggests a breeding area as far south as 5°N. Whether or not Vladykov's modification of Schmidt's hypothesis is correct, the eel seems to breed at least 3000 km south of Nova Scotia. Because of this great distance, it seemed important to establish how the breeding area is located by the eels.

There are several ways in which the eels could reach the breeding area, e.g. random search, olfactory mechanisms, following salinity or temperature gradients, joining the deep current running counter to the Gulf Stream (Volkman, 1962), following the coastline, and so on; but critical analysis of these methods suggests that they are not likely to be used by the eels (Miles, MS, 1967).

Since a great variety of animals can orient themselves using the sun during the day, or the stars at night, including fishes (Hasler, 1966), it was proposed to test whether the eel also is capable of this celestial orientation. The maintenance of a southerly orientation would allow the eel to reach the general vicinity of the breeding area (Verwey, 1949).

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Printed in Canada.

MATERIALS AND METHODS

Tests to determine the orientational behaviour of adult eels were performed on the roof of the Biology Building (20 m high) on the campus of Dalhousie University, Halifax, N.S., in the fall and winter of 1964, 1965, and 1966. Adult eels on their seaward spawning migration were used in most of the tests; nonmigrants were used in the remainder. Eels were obtained from Ketch Harbour Brook, the Medway River, and the estuary of the Miramichi River (Fig. 1). Their coloration and the time and manner in which

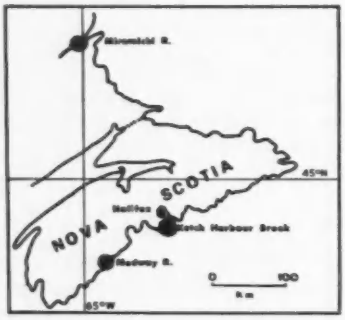

FIG. 1. Nova Scotia and adjacent New Brunswick showing the locations of the rivers from which eels were caught.

they were caught were the criteria used to determine whether the eels were migrants (Bertin, 1956); the eels were all about 30 cm long. A total of 95 eels were tested in 10 tests; they were maintained in fresh water in an open tank, the walls of which were sufficiently high to allow a view only of the sky. The eels were tested usually within a week of being captured, and were not fed while in captivity. Examination of the eels after testing showed that all the eels tested were females.

Five categories of tests were performed, and are described in detail below. In all tests orientational behaviour was recorded on 16-mm ciné film (Eastman Panchromatic Plus-X). One exposure was made every $7\frac{1}{2}$ min both day and night. Four 350-w photoflood lamps, arranged symmetrically about the tank, lit the scene for about 1 sec for each exposure.

Circular tanks of two sizes were employed in the tests. One, used in 1964, was 2 × 0.7 m deep, and was set on the ground. The camera was centralized 2.2 m above the tank, and the lamps were arranged around the rim of the tank. The other tank was 1 × 0.3 m deep. The base of this tank was 6-mm transparent "Perspex," and the wall was of resinbonded fibreglass painted white. This tank was mounted on a box 1.2 m high with 1-m sides with the top open to provide a view through the transparent base of the tank. The camera was centralized 1 m beneath the tank, and the four lamps were arranged beneath the tank.

Tests were performed either with synthetic salt water or with fresh water to a depth of 15 cm. Salt water was made by dissolving Rila Marine Mix (Rila Corp., Tea Neck, New Jersey) to a concentration of 32‰. The tanks were located in such positions that the skyward view was unobstructed by any objects, except the tripod legs and camera over the larger tank, unless this view was obscured for experimental purposes.

A single test of the behaviour of eels in a particular set of conditions lasted at least 5 days. Usually five eels were tested in the larger tank at any one time, and two or three in the smaller tank. Eels that had not been previously tested were placed in the tank at the start of the test, and these were replaced with other untested eels every 3–4 days (except in tests 4, 8, 9, and 10, when only two eels were tested throughout each test, as a result of a shortage of eels); in this way a fair sample of eels was used in each test. The tank and camera were rotated at intervals in all tests; they were rotated 180° half-way through test no. 1 (1964); in 1965 and in 1966 they were turned through 90° each evening, the direction being fixed for any particular test, but variable among tests. Daily records of hours of sun and amount of cloud cover were obtained from the Canada Department of Transport, Meteorological Branch, when required.

Analysis of Results

Processed film was viewed with a table model film editor. The direction of orientation of each eel in each frame was estimated to the nearest 45° division of the compass and assigned to one division. An eel was considered to be oriented in the direction that its head was pointed, rather than by the "average" body direction or any other criterion. The numbers of eels assigned to each of the eight compass points for each day (dawn to dusk), or for each night (dusk to dawn) were totalled; each set of eight totals will be called henceforth a *semi-day record*.

To estimate the direction of orientation in any test, two calculations were performed.

(*a*) All the semi-day records of the test were summed; this set of eight totals will henceforth be called a *summary*. This summary was treated as a vector diagram, and the vector direction and length calculated. The possibility exists that an eel may not act as an individual but be influenced to some extent by the other eels and tend to become aligned in the same direction as some of the other eels. To take into account the possibility that the eels interact, all summaries were divided by the number of eels present in the tank at any one time, before any further calculations were made.

(*b*) The direction of orientation for each semi-day was also calculated, by treating each semi-day record as a vector diagram. All the semi-day directions or azimuths of a test were plotted on a circular diagram, and each semi-day azimuth considered to have equal strength as a vector. This set of azimuths will henceforth be called an *azimuth summary*. The azimuth sum-

mary was resolved vectorially, and the direction of orientation of the eels in the test calculated.

Since there was no reason to consider the direction of orientation based either on the summary or on the azimuth summary to be a true representation of the orientation of the eels, the direction of orientation was taken to be mid-way between the two directions calculated.

The Raleigh test was used to determine whether the orientation calculated was due to chance (Schmidt-Koenig, 1961; Batschelet, 1965; see Fig. 2). The test statistic, Z, is calculated thus:

$$Z = V^2/n$$

where V = the vector length, n = the number of observations. In the case of the summaries, V must be corrected because the data have been grouped within intervals of 45°; if β is half the group interval (i.e. 22.5°), measured in radians, the vector length should be corrected thus:

$$V = V_u \cdot \beta/\sin \beta$$
$$= V_u \cdot 1.0262$$

where V_u = the uncorrected vector length.

For eels to be considered to be orientating themselves in a nonrandom manner in any test, values of $p \leq .05$ must be recorded in both calculations (*a*) and (*b*) above.

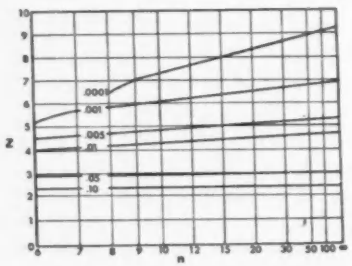

FIG. 2. The critical values of the test statistic Z for different values of n (number of observation). (From Schmidt-Koenig, 1961.)

In a similar fashion not only could whole tests, but also parts of tests, be analysed. Thus the nighttime or daytime orientation, or the orientation during those semi-days in which certain weather conditions prevailed, could be calculated, by considering the appropriate semi-days.

TYPES OF TEST

Ten tests were performed and are classified into six categories; each category consists of one or more tests under similar conditions. A resumé of the 10 tests is shown in Table I.

TABLE I. Summary of the conditions of the tests performed.

Test no.	Year	Test conditions	Test water	Tank diam (*m*)	Source of eels[a]	No. of eels	Migratory status	No. of semi-days
1	1964	Roof; open	Salt	2	K.H.	40	Migrant	31
2	1965	"	"	1	K.H.	7		33
3	1966	"	"	1	Medw.	18	"	17
4	1965	"	Fresh	1	K.H.	2	"	17
5	1965	"	Salt	1	Mira.	6	Nonmigrant	24
6	1965	"	Fresh	1	Mira.	10	"	19
7	1966	"	Salt	1	Medw.	6	"	10
8	1965	Opaque cover	"	1	K.H.	2	Migrant	25
9	1965	Total dark	"	1	"	2	"	14
10	1965	Light from NE	"	1	"	2	"	13

[a]K.H. = Ketch Harbour Brook; Medw. = Medway River; Mira. = Miramichi River.

(*a*) *Migrants in salt water.*— These tests (No. 1–3) were performed in 1964, 1965, and 1966.

(*b*) *Migrants in fresh water.*— Test No. 4.

(*c*) *Nonmigrants.*— These eels were tested both in fresh water (No. 6) and in salt water (No. 5 and 7).

(*d*) *Migrants in salt water; tank covered.*— The tank was covered with two layers of black polyethylene film which was to the eye totally opaque to sunlight. However, because of the construction of the box on which the tank was set, indirect light could filter in, although no direct view of the sky or surroundings was available (No. 8).

(*e*) *Migrants in salt water; dark room.*— Eels from the outdoor holding tank were tested with no acclimation period in a basement room without windows or other access to natural light. Apart from the intermittent illumination of the flood lamps necessary for the exposure of the film, no light was present (No. 9).

(*f*) *Migrants in salt water; incandescent lighting.*— This test (No. 10) was performed in the same room as test No. 9 (*e* above). A 100-w lamp was situated northeast 1 m from the wall of the tank and 0.6 m above the level of the top of the wall of the tank. The lighting schedule was the same as the then current daylight outside.

RESULTS

The results of the 10 tests are considered in the same categories as in the previous section. A summary of the results is shown in Fig. 3. The statistical analysis of the azimuth summary data almost always gave a lower level of significance than the analysis of the summary of a test. The reason for this is likely that each 7½-min observation was not entirely independent of previous observations, whereas the observation of the direction of the eels in one semi-day was very unlikely to be influenced by the behaviour of the eels during previous semi-days; the Raleigh test would be valid only when each observation is independent of all other observations. Consequently, the eels were considered to be orienting in a nonrandom manner only when the azimuth summary gave a value of $p < .05$.

In many cases, the analyses were made for the purposes of comparison only; the values of Z obtained under different conditions were compared in an attempt to relate the orientational behaviour of the eels to the prevailing external conditions. The values of Z can be compared providing the numbers of observations in the parts being compared do not differ greatly.

The two directions, obtained from each of the two methods of analysis in any test, were similar.

Results of the Types of Test (Shown in Fig. 3 and Table II)

(*a*) *Migrants in salt water.*— In all three tests the eels oriented themselves nonrandomly in a southerly direction. Two of the three tests gave higher Z values for nights than for days. The first two tests were analysed

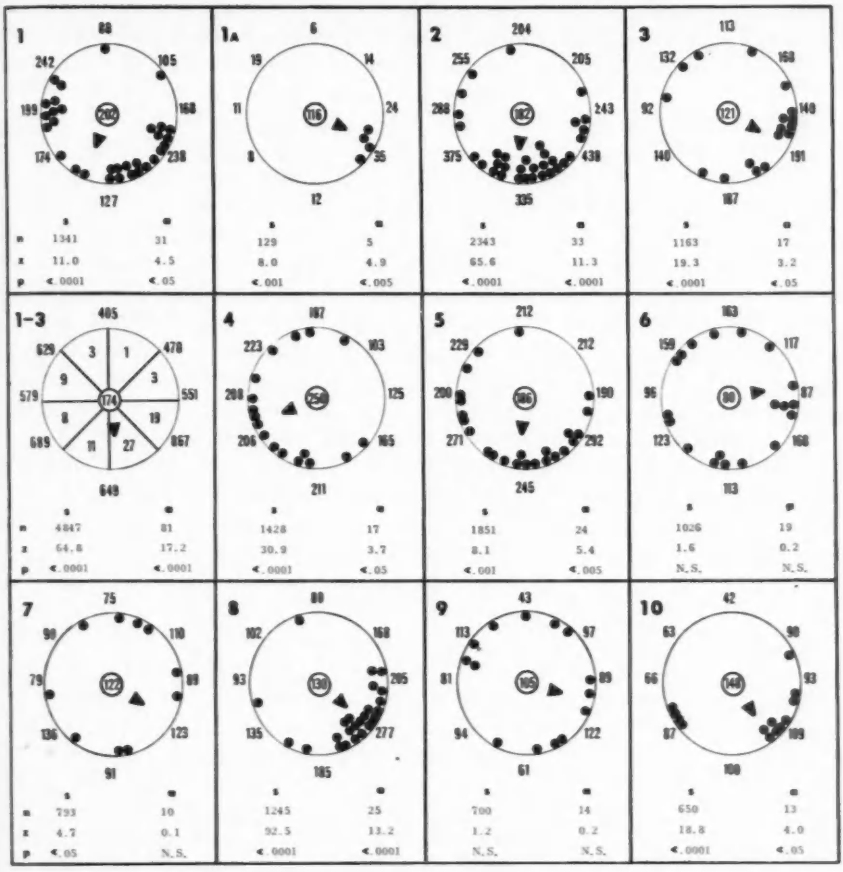

FIG. 3. Summary of the results of the 10 tests performed. Each section represents one test (except those sections marked 1A and 1–3). The eight numbers around the edge of the circle represent the *summary* of that test, and the solid circles represent the *azimuth summary*. The number in the centre is the orientation of the eels in degrees (also shown by the solid arrowhead). The number of observations (n), the Z values, and the level of probability (p) of both the summary (s) and the azimuth summary (a) are shown. The section marked 1A represents that part of test No. 1 that was performed under constant cloud. Tests 1–3, being performed under the same conditions in three consecutive years, are summarized; the azimuth summary is not shown as solid circles but for clarity as the number of circles in each octant.

for the effects of different weather conditions; Z values were higher in the summaries of those days with less than 6 hr of sun than of those days with more; higher Z values generally accompanied increasing cover by cloud.

Part of test No. 1 was analysed separately. On one morning the sky was totally overcast; five fresh eels were put in the large tank and filmed. Totally

TABLE II. Summary of the results of the first five tests, which were analysed for the orientation of the eels by day and by night, under three different levels of cloud cover, and during days with less than and more than 6 hr of sunshine.

Test no.		Days		Nights		Cloud cover 0/10–4/10		Cloud cover 5/10–9/10		Cloud cover 10/10		Sun <6 hr		Sun >6 hr	
		199[a]		*205*		*205*		*246*		*134*		*210*		*172*	
		s[b]	*a*	*s*	*a*	*s*	*a*	*s*	*a*	*s*	*a*	*s*	*s*	*s*	*a*
1	*n*	542	15	802	16	491	12	603	10	246	9	254	8	286	7
	Z	3.6	2.0	7.0	2.8	4.7	2.3	13.2	1.3	7.4	4.4	5.4	1.2	0.7	0.8
	p	<.05	NS	<.01	NS	<.01	NS	<.0001	NS	<.001	<.01	<.005	NS	NS	NS
		169		*192*		*220*		*190*		*163*		*148*		*203*	
2	*n*	973	19	1369	14	363	6	1050	17	923	10	668	12	305	7
	Z	28.6	4.9	34.5	7.7	3.4	1.3	32.7	5.5	37.8	6.3	19.2	3.5	18.7	2.6
	p	<.0001	<.01	<.0001	<.001	<.05	NS	<.0001	<.005	<.0001	<.001	<.0001	<.05	<.0001	NS
		132		*82*											
3	*n*	488	8	673	9										
	Z	22.0	2.4	7.8	2.8										
	p	<.05	NS	<.001	NS										
		216		*255*											
4	*n*	502	8	926	9										
	Z	9.1	1.4	23.3	2.3										
	p	<.001	NS	<.0001	NS										
		148		*206*											
5	*n*	817	12	1034	12										
	Z	2.6	1.9	8.2	5.1										
	p	NS	NS	<.001	<.005										

[a]The numbers in italics refer to the direction of orientation in degrees of the eels under the particular conditions.
[b]*s* = summary data; *a* = azimuth summary data; *n* = number of observations; Z = the test statistic used to determine *p*.

overcast weather continued for 2 days, so that 5 semi-days of data were collected. These eels oriented themselves nonrandomly roughly southeast with a high degree of reliability.

(*b*) *Migrants in fresh water.*— In this test (No. 4) the orientation was nonrandom, in a southwest direction. The summary Z value for nights was higher than the value for days.

(*c*) *Nonmigrants.*— Three tests were performed, two in salt water (No. 5 and 7) and one in fresh water (No. 6). The results were not consistent. In one test in salt water the orientation was roughly south, and was nonrandom; the summary value of Z for nights was higher than the value for days. In another similar test, the alignment of the eels was random. A further test in fresh water gave a random alignment. However, if the data from test numbers 6 and 7 are summed (over a total of 20 semi-days), the Z value of the azimuth summary data is 0.31, $p > 0.05$, i.e. the orientation was random.

(*d*) *Migrants in salt water; tank covered with black polyethylene film.*— In this test (No. 8) the orientation was strongly southeast.

(*e*) *Migrants in salt water; total darkness.*— In this test the alignment of the eels was random.

(*f*) *Migrants in salt water; incandescent lighting.*— In this test the orientation of the eels was strongly to the southeast.

DISCUSSION

The results of the first three tests indicate that the orientation of migrating eels in salt water, in a situation in which they can see only the sky, is southward. That the eels did this when tested in tanks both 1 and 2 m in diam, and in 3 successive years, suggests strongly that this orientation was not the result of some bias provided in the apparatus. If the eels showed this orientation in the sea also, it would enable them to reach the general locality of their breeding area from Nova Scotia. Since the direction of migration is more or less south, it is not possible to tell whether the eels have a sense of direction alone, or whether they have a sense of geographical position which would allow the animal to move along the great circle joining the starting and finishing points (as discussed by Deelder, 1949). Additional cues might operate to allow for serious lateral drift, if there were any, and to stop the migration when the breeding area has been reached.

Several control tests were performed. The eels aligned themselves randomly in two tests with apparent nonmigrants (No. 6 and 7) whereas in another such test (No. 5) a significant southward orientation was shown. This instance of orientation in apparent nonmigrants could have resulted from the inadvertent presence of some migrants in the group. The colour of migrants is somewhat variable, and since the nonmigrants were collected from traps in early September there is a possibility that migrants could also have entered the traps; migrants would have been passing down the river at that time. It is unlikely that nonmigrants would exhibit a capacity for orientation, for there seems to be no

function for such behaviour. In fact, when the results of tests 6 and 7 were summed, giving data for 20 semi-days, the eels were shown to be still orienting randomly.

Another test (No. 4 — migrants in freshwater) gave a result that was not easily explained. In this test the orientation of the eels was southwest. The general direction of Ketch Harbour Brook, from which these eels had been collected, is south, and it is possible that such orientation would allow the eels to leave the system. How this orientation is acquired is not known. It is possible that the eels, finding themselves moving downstream on their migratory journey, learn the direction in which they are travelling, and so expedite the process. This explanation could be checked by testing eels leaving a river that flows in another quite different direction.

In one control test (No. 9) in which migrants were tested in darkness, the alignment of the eels was random with a Z value lower than in any other test. The result of this test suggests that eels are not capable of orienting themselves in the absence of a view of the sky. However, in test No. 8 (in which the tank was covered with opaque polyethylene film), orientation was southeast with a degree of significance higher than that of any other test. As explained before, indirect daylight could reach the eels through spaces in the walls of the box supporting the tank, but the sky was totally obscured. Most other animals that are able to orient themselves in a certain compass direction are unable to do so when their view of the sky is obscured (e.g. Schmidt-Koenig, 1965; Hasler, 1966).

Another test that resulted in significant orientation when the sky was obscured was No. 10. The tank was set in a room with no windows, and an incandescent lamp was situated northeast of the tank. Under these circumstances the orientation of the eels was to the southeast. Work with several groups of animals has shown that many that are capable of celestial orientation are able to orient themselves appropriately using a fixed light in place of the sun (Hoffman, 1965; Schmidt-Koenig, 1965; Hasler, 1966). If the orientation of the organism is normally to the south, since the light is fixed, the organism would keep the light to its left in the morning and to its right in the afternoon, no matter what the geographical direction of the light. In the present test, despite the lamp being to the northeast, the eels oriented themselves to the southeast, as in other tests. This test, and the test with the tank covered, suggests that the orientation of the eels is, at least under those conditions, noncelestial. It seems, however, that the eels do need the diurnal light–dark rhythm for orientation, for in total darkness they are disoriented. The analysis of orientation of eels under different conditions of cloud cover suggests this also.

The alignment of the eels was generally less random with increasing cloud cover and with decreasing number of hours of sun. The part of test No. 1 (continuous overcast) that was analysed separately also indicated that increased cloud cover, even overcast, does not interfere with the orientation of the eels. This conclusion contradicts most results of work with fish, am-

phibians, and birds; these animals are unable to orient themselves when the sun is obscured, unless only temporarily when fixed reference points may sometimes be used to maintain the orientation. It is possible that the eels used reference points in the tests (although they could not do so at sea); they could refer to some mark on the wall of the tank which could be used when the sun was obscured. But this seems unlikely as the only marks were small blemishes and so on, and there were a great many of these. The procedure of rotating the tank eliminated the possibility of features being used for long periods of time. The observation that the orientation of eels was generally better when there was more cloud might have been the result of a tendency of the eels to remain in the shaded part of the tank; on clear days the alignment of the eels would therefore tend to be more scattered as the eels could not often remain in the shade and orient themselves at the same time southwards.

Nevertheless, the evidence indicates that the migrating adult American eel is capable of noncelestial orientation. Groot (1965) concluded that smolts of the sockeye salmon (*Oncorhynchus nerka*) on their seaward migration were also capable of noncelestial orientation.

Celestial orientation of the type demonstrated by many organisms would be subject to severe limitations in the ocean. During the day the azimuth or the altitude, or both, of the sun seem to be used in orientation. The precise mechanism of nighttime orientation is at present not known, but seems to depend on star patterns rather than star azimuths (Matthews, 1963; Emlen, 1967). In the ocean there are factors which do not apply on land. The surface of the ocean is almost invariably disturbed, and sunlight penetrates only to about 300 m; the image of the sun would he visible to a much lesser depth.

However, even if an indication of the position of the sun was available to the fish (Whitney, 1941; Henderson, 1963), it would not be during cloudy periods. Furthermore, it does not seem possible that the star pattern would be visible at any time beneath the surface of the ocean. The fish would not be able to hold its position with any certainty, let alone continue migrating, by referring to fixed reference points, as some birds do when adverse conditions prevail.

Off Nova Scotia (45°N, 60°W) in September there is 9/10 cloud cover 30% of the time; the average cloud cover is 5/10. In about 2 months an eel will have reached roughly 35°N, 60°W (Schmidt, 1924) where the average cloud cover will still be 5/10. Farther south the cloud cover is less, being about 4/10 at 20°N, 60°W in October to January. Put another way, this means that the sun is visible at most only half the time for the first 2 months of the eels' journey south. If we suppose eels cannot orient themselves at night, or when the sun is not visible, then they could orient themselves less than one-quarter of the time while in the northern latitudes, and only slightly more than that closer to the breeding area.

The same problem exists for the returning Pacific salmon, and Hasler (1966, p. 122–123) says that:

"... because of the prolonged cloudy periods which often predominante in the northern reaches of the oceans where the salmon live, we must yet discover augmentative sensory and physiological abilities to account for the remarkable migratory feat the salmon appears to perform."

In the light of the above, the possession by eels of noncelestial orientation would be of considerable advantage. Furthermore, no records have come to my notice of eels being caught more than a few kilometers from shore. This would suggest that eels swim deeper than the operational depth of most fishing gear.

The orientation of the eels necessary to bring them to the breeding area need not necessarily be very precise, for Saila and Shappy (1963; see also Patten, 1964a, b; Saila and Shappy, 1964) have shown in a mathematical model that salmon returning to their natal streams do not need a true navigational ability or even precise directional orientation. It seems safe, therefore, to assume that the orientation demonstrated by migrating eels would allow them to reach the general area of their breeding ground.

Note added in proof: Experiments similar to those just described were carried out in Gloucestershire, England, using European eels, but the eels did not show any significant orientation. The eels were migrants from a tributary of the River Severn.

Two tests were performed; in the first the eels were tested in sea water with the tank open to the sky; in the second the tank was covered with opaque polyethylene film. In neither test, nor in the summed data of both tests, did the eels show any significant orientation.

Unless further tests are carried out, no explanation can be offered for this difference in behaviour.

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REFERENCES

BATSCHELET, E. 1965. Statistical methods for the analysis of problems in animal orientation and certain biological rhythms. A.I.B.S. monograph. 57 p.

BERTIN, L. 1956. Eels; a biological study. Cleaver-Hulme, London. 192 p.

DEELDER, C. L. 1949. On the orientation of migrating animals. Bijdragen Dierkunde, **28**: 503.

EMLEN, S. T. 1967. Migratory orientation in the Indigo Bunting, *Passerina cyanea*. Part I: Evidence for the use of celestial clues. Auk, **84**: 309–342.

GROOT, C. 1965. On the orientation of young sockeye salmon (*Oncorhynchus nerka*) during their seaward migration out of lakes. Behaviour, Suppl. XIV. 198 p.

HASLER, A. D. 1966. Underwater guideposts. University Wisconsin Press, Madison, Wis. 155 p.

HENDERSON, H. F. 1963. Orientation of pelagic fishes. I. Optical problems. II. Sonic tracking. Dissertation Abstr., **24**: 2626.

HOFFMAN, K. 1965. Clock mechanisms in celestial orientation of animals. *In* J. Aschoff [ed.] Circadian clocks. North Holland Publ. Co. p. 426–441.

MATTHEWS, G. V. T. 1963. The astronomical basis of 'nonsense' orientation. Proc. XIII Intern. Ornith. Congr. p. 415–429.

MILES, S. G. MS, 1967. The orientation of the adult American eel (*Anguilla rostrata* LeSueur). Ph.D. Thesis. Dalhousie University, Halifax, N.S.

PATTEN, B. C. 1964a. The rational decision process in salmon migration. J. Conseil, Conseil Perm. Intern. Exploration Mer, **28**: 410–417.

1964b. Letter to the Editor: Salmon migration. Ibid., **28**: 443–444.

SAILA, S. B., AND R. A. SHAPPY. 1963. Random movement and orientation in salmon. Ibid., **28**: 153–166.

1964. Letter to the Editor: Salmon migration. Ibid., **28**: 440–443.

SCHMIDT, J. 1924. The breeding place of the eel. Smithsonian Rept., **1924**: 279–316.

SCHMIDT-KOENIG, K. 1961. Die Sonne als Kompass in Heimorientierungssystem der Breiftauben. Z. Tierphysiol. Tierernachs. Futtermittelk., **18**: 301–331.

1965. Current problems in bird orientation. *In* D. S. Lehrman, R. A. Hind, and E. Shaw [ed.] Advances in the study of behaviour, Vol. I. Academic Press, Inc., New York. p. 217–278.

VERWEY, J. 1949. Migration in birds and fishes. Bijdragen Dierkunde, **28**: 503.

VLADYKOV, V. D. 1964. Quest for the true breeding area of the American eel (*Anguilla rostrata* LeSueur). J. Fish. Res. Bd. Canada, **21**: 1523–1530.

VOLKMAN, K. 1962. Deep current observations in the western North Atlantic. Deep-Sea Res., **9**: 493–500.

WHITNEY, L. V. 1941. The angular distribution of characteristic diffuse light in natural waters. J. Marine Res., **4**: 122–131.

Dissolution of Fish Muscle Homogenates by Lysolecithin[1]

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ABSTRACT

On incubating skeletal muscle homogenates from rainbow trout with lysolecithin (LL) and comparing them with homogenates of the same muscle without added LL, and after centrifuging the mixtures, it was found that the N content of the supernatant solution of the homogenate containing LL was about 20% higher than that of the homogenate without LL. Increases close to maximum in N content of the supernatant solution were found to occur at a concentration of about 4 mg LL per ml of incubation medium containing 100 mg muscle in 3.0 ml of 0.9% NaCl at a *p*H of 6.0–8.0 and at about 35 C for a period of 1 hr. Snake venom phospholipase A added to muscle homogenate showed no solubilizing activity and α-tocopherol acetate and cortisol showed irregular stimulation. It was concluded that LL exerts a solubilizing action on fish muscle homogenates.

INTRODUCTION

Considerable attention has been focused on storage changes in fish muscle (Dyer and Morton, 1956; Lovern and Olley, 1962; Bligh and Scott, 1966) including lipid hydrolysis and phospholipid loss (Olley and Lovern, 1960) and drip formation (Tarr, 1941; Tomlinson et al., 1967). Whereas a great deal of work has been done on the contribution made towards these changes by free fatty acids formed as a result of phospholipase activity (free fatty acid–protein interaction in relation to protein denaturation), very little work has been done on the possible role of other products of degradation (Olley et al., 1962; Ackman, 1967; Dyer, 1967). Since phospholipase A is present in fish muscle tissue (Jonas and Bilinski, 1967a, b), it was only logical to assume that lysolecithin (LL) was being formed in fish muscle and could produce changes not attributed to it hitherto.

Phospholipase A activity with the formation of LL occurs very widely in animal tissues such as nerves, brain, liver, kidney, pancreas, stomach, intestine, testis, and skeletal muscle (Gallai-Hatchard and Thompson, 1965; McArdle et al., 1960; Ottolenghi and Bernheim, 1961). As a hemolytic and surface active agent (Marples et al., 1959; Thompson, 1964; Klibanski and de Vries, 1964) LL has implications in immune hemolysis, hypersensitivity reactions in localized inflammation, and increased vascular permeability after tissue damage (Högberg and Unväs, 1957; Keller, 1962; Mellors and Tappel, 1967). A clearer picture of the various biochemical properties and involvement of LL in vivo could emerge from a consideration of its presence

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in connection with the tissues where it is found in the highest concentration, and of some of its coexisting lipids and enzymes and their reactions. Thus, chromaffin granules of the adrenal medulla are known to contain 17% of their lipid P as LL whereas mitochondria and microsomes contain about 2%, and lysosomes are known to contain higher amounts (Blaschko et al., 1967; Mellors and Tappel, 1967). Mast cells are also known to contain LL (Högberg and Unväs, 1957). The presence and properties of LL in such tissues and organelles suggest that it could have cytolytic effects in fish muscle as well. The following is a report on one phase of this work. Though two different varieties of phospholipase A are known to exist (Waite and van Deenen, 1967), the term phospholipase A is used in the text without specific reference to either variety.

EXPERIMENTAL

Fish

Two-year-old rainbow trout (*Salmo gairdneri*) used in this experiment were obtained from a commercial hatchery and kept in a freshwater aquarium on a ration of pelleted food.

Preliminary experiments showed small variations in the amount of dissolution that occurred in the homogenates of samples from different fish. Some variation in muscle protein due to sex maturation has been observed in salmon (MacLeod et al., 1960) and it is quite possible that similar changes could occur in rainbow trout. For this reason, two to three fish were killed and their muscle pooled in some instances.

Chemicals

Lysolecithin and other chemicals used were the purest commercial grades available.

Tissue Preparation and Incubation

Skeletal muscle from the dorsal area of the fish, free from skin and bone, was cut into small pieces. Muscle (100 mg) was introduced into a test tube and homogenized with 0.9% NaCl for 1 min using a Potter-Elvehjem type homogenizer connected to an automatic timing device. The required reagents were then added and, mixed; the air was replaced by nitrogen; and the tubes were then stoppered and incubated at 35 C for 1 hr. The tubes were cooled in ice at the end of the incubation period; centrifuged at 1600 $\times$ *g* for 3 min; and the supernatant solution was decanted into Kjeldahl flasks. NaCl (5.0 ml 0.9%) was added to the residue in the test tubes, mixed, and the tubes were then recentrifuged for 3 min to remove any soluble material in the residue. The supernatant solution was added to the previous solution in the Kjeldahl flask. Nitrogen was determined by the micro-Kjeldahl method (Steyermark, 1951) both in the aqueous supernatant solution and in the insoluble residue.

The incubation mixture consisted of 100 mg skeletal muscle homogenate and 12 mg of LL with or without the test reagent in a total volume of 3.0 ml of 0.9% NaCl. Control blanks with muscle only, muscle and test reagent, and muscle and LL were also carried out for each test where a test reagent other than LL alone was used.

RESULTS

The nitrogen value for LL was deducted from the nitrogen value of the supernatant solution (Table I–III) wherever LL was added to the muscle. Since it has been suggested that some preparations of LL could contain entrapped snake venom (Cohen et al., 1967), the LL used in this experiment

TABLE I. Effect of lysolecithin on rainbow trout skeletal muscle homogenate.

	% nitrogen in the supernatant			
	Muscle only		Muscle + LL	
No. of samples	Mean	Range	Mean	Range
15	31.0±0.51*	27.5–33.2	51.2±0.82*	46.0–56.8

*Mean and standard error of the mean $P < 0.01$.

TABLE II. Effect of time of incubation and *p*H on the action of lysolecithin on rainbow trout skeletal muscle.

	% nitrogen in the supernatant						Mean % increase in N due to addition of LL
	Muscle only			Muscle + LL			
Changing factors	Mean	Range	No. of expt.	Mean	Range	No. of expt.	
Time (*hr*)							
0.5	29.5	28.0–31.0	3	54.0	49.6–58.0	3	24.5
1.0	31.5	29.0–33.1	3	54.9	54.7–55.4	3	23.4
2.0	32.6	31.0–33.8	3	53.8	50.5–58.5	3	20.0
4.0	31.4	30.6–32.6	3	55.9	54.5–57.3	2	24.5
*p*H							
5.0	22.4	20.8–24.3	3	35.9	33.0–38.8	3	13.5
6.0	32.9	28.2–37.2	3	53.1	51.6–55.1	3	20.2
7.0	40.0	39.6–40.4	3	60.3	56.6–64.0	2	20.3
8.0	40.0	39.2–40.8	2	60.9	59.5–61.9	3	20.9
9.0	48.1	47.2–49.0	2	60.9	60.6–61.8	2	12.8

TABLE III. Effect of snake venom on the lysolecithin action in skeletal muscle homogenate from rainbow trout.

Changing factors	% nitrogen in the supernatant		
	Mean	Range	No. of expt.
Skeletal muscle only	32.4	32.0–32.8	3
Skeletal muscle + 0.1 μg snake venom	32.3	31.8–33.3	3
Skeletal muscle + 1.0 μg snake venom	31.0	28.0–32.7	3
Skeletal muscle + 10.0 μg snake venom	31.1	27.2–32.9	3
Skeletal muscle + 0.1 μg snake venom + LL	55.2	54.4–56.2	3
Skeletal muscle + 1.0 μg snake venom + LL	55.3	54.4–56.5	3
Skeletal muscle + 10.0 μg snake venom + LL	56.0	52.0–58.4	3
Skeletal muscle + LL	49.7	47.0–54.6	3

was assayed for phospholipase A activity by employing ^{14}C-labelled lecithin as described previously (Jonas and Bilinski, 1967a, b). It showed an activity that gave approximately 90 mμmoles of LL per hour for a 12-mg sample (the amount usually used in the incubation medium). In an experiment where ordinary skeletal muscle was compared with lateral line muscle in its reaction to LL, no difference was observed between the two tissues.

It was thought possible that the LL used could have a proteolytic action instead of a cytolytic action. Proteolytic activity could degrade fish protein whether the protein was a heated or unheated preparation. The same quantities of LL were added to a heated and unheated homogenate and incubated in the usual way. The boiled preparation showed no N increase in the supernatant solution proving that LL had no primary proteolytic action on boiled fish muscle.

Fifteen samples of skeletal muscle showed an increase of ≈20% in the supernatant solution N content due to the addition of LL (Table I). The above results when subjected to statistical analysis by applying the *t*-test showed a highly significant increase ($P < 0.01$) in supernate nitrogen after the incubation of the muscle in the presence of LL.

EFFECT OF TEMPERATURE

The solubilizing effect of LL was dependent on temperature. From 0 to 20 C no reaction was detected, whereas at 35 C ≈20% increase in N in the supernatant was obtained.

EFFECT OF TIME

Increasing the incubation time from 0.5 hr to 1, 2, and 4 hr showed very little change in the supernatant N content (Table III).

Effect of LL Concentration

Increasing the concentration of LL in the incubation medium from 0.2 mg to 4.0, 6.0, and 8.0 mg/ml showed an approximate increase of 14% for 2 mg/ml and 20% for 4 mg/ml in the supernatant nitrogen. At 8.0 mg/ml the increase was ≈26% (Fig. 1).

Effect of *p*H

Influence of *p*H on the action of LL is shown in Table II. At *p*H 5.0 and 9.0, ≈13% increase in the N content of the supernatant was found. At *p*H 5.0 a reduced protein solubility was associated with reduced solubilization, whereas at *p*H 9.0 the seemingly larger increase was reduced by the greater solubility of protein in the strongly alkaline solution for the muscle blank. From *p*H 6.0 to 8.0 the increase in the supernatant N persisted at the 20% mark. The *p*H of 0.9% NaCl was found to be 6.0.

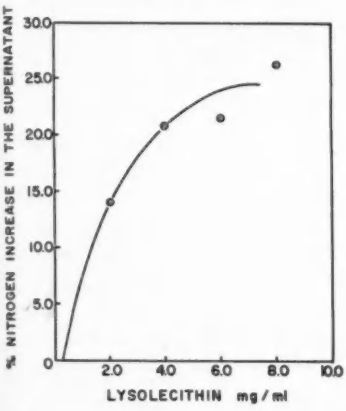

FIG. 1. Effect of increasing lysolecithin concentration in the incubation medium.

Effect of Snake (*Crotalus adamanteus*) Venom

Since both the skeletal muscle and the LL used have very small phospholipase A activities, it was determined to what extent a very active phospholipase A enzyme preparation could cause solubilization in a skeletal muscle homogenate. For this purpose, the highly active phospholipase A of snake venom was used in three different concentrations (0.1, 1.0, and 10 μg). No increase in the supernatant N was observed in the venom and muscle mixture. A small increase of 5% was noted in the mixture containing LL + venom + muscle. It was clear from these results that solubilization of skeletal muscle homogenate attributed to LL was not being caused by phospholipase A in this experiment.

Effect of Cortisol and α-Tocopherol Acetate

Since cortisol and α-tocopherol are naturally occurring substances in many types of fish (Fagerlund, 1967; Ackman and Cormier, 1967; Ackman,

1967; Olcott, 1967), varying with season and maturity, it was determined whether they had any effect on the solubilizing property of LL. Three different concentrations of cortisol were used: 0.2, 2.0, and 20 μg per ml of incubation medium. No change due to cortisol was observed in the supernatant N content.

When α-tocopherol acetate was used (47 mg/g N), ≈4% increase in the supernatant N was obtained.

DISCUSSION

Though some effects of LL on different kinds of tissue slices are known (McArdle et al., 1960), its solubilizing effect in skeletal muscle tissue homogenate has not been demonstrated before. Although cytolysis alone could increase the protein nitrogen of the supernate, it is known that intracellular proteolytic enzymes released by disruption of cells (Marples et al., 1959) could also be active in the solution. Table II shows the effect of *p*H on LL action. This is different from that obtained for lecithinase activity and phospholipase A activity in previous experiments (Bilinski and Jonas, 1966; Jonas and Bilinski, 1967a, b). Whereas LL action is independent of *p*H between 6.0 and 8.0, phospholipase activity is dependent on *p*H and changes with *p*H in this range. Very little increase in the supernatant N content was obtained between 0.5 and 4 hr. Thus, *p*H and incubation time on the action of LL strongly suggest that the primary LL reaction is possibly a physico-chemical rather than an enzymatic phenomenon. The reaction of snake venom alone on muscle homogenate proved beyond doubt that the solubilizing action in skeletal muscle homogenate in this experiment was not a result of phospholipase A activity. However, the small increase of N in the supernatant liquid suggests that phospholipase A could possibly stimulate the action of LL. Though cortisol had no stimulating effect and a small increase was obtained with α-tocopherol acetate, it is possible that optimal conditions for their effects may have been different.

Only trace amounts of LL have been reported in fish muscle and its concentration does not increase in cold-stored fish muscle (Lovern et al., 1959; Bligh, 1961; Olley et al., 1962; Yurkowski and Brockerhoff, 1965). The present experiment has also shown no LL activity below 20 C and no solubilizing activity due to phospholipase A in the course of 1 hr. However, cold-stored muscle kept for many weeks or months may continue to have enzyme reactions taking place at too low a rate to be detected by conventional methods. An increase in glycerylphosphorylcholine and free fatty acid in cold-stored fish muscle, and evidence for phospholipase A activity in fish skeletal muscle (Jonas and Bilinski, 1967a, b) have already been found. LL could be present where phospholipase A activity exists. Thus it is considered possible that one of the factors influencing the formation of drip in cold-stored muscle could be implicated in the solubilizing action of LL, especially in commercially caught fish which may have sustained body injury because of the increased phospholipase A activity, resulting in increased LL formation in the damaged tissues (Högberg and Unväs, 1957; Mellors and Tappel, 1967).

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REFERENCES

ACKMAN, R. G. 1967. The influence of lipids on fish quality. J. Food Technol., **2**: 169–181.

ACKMAN, R. G., AND M. G. CORMIER. 1967. α-Tocopherol in some Atlantic fish and shellfish with particular reference to live-holding without food. J. Fish. Res. Bd. Canada, **24**: 357–373.

BILINSKI, E., AND R. E. E. JONAS. 1966. Lecithinase activity in the muscle of rainbow trout. Ibid., **23**: 207–220.

BLASCHKO, H., H. FIREMARK, A. D. SMITH, AND H. WINKLER. 1967. Lipids of the adrenal medulla. Lysolecithin, a characteristic constituent of chromaffin granules. Biochem. J., **104**: 545–549.

BLIGH, E. G. 1961. Lipid hydrolysis in frozen cod muscle. Ibid., **18**: 143–145.

BLIGH, E. G., AND M. A. SCOTT. 1966. Lipids of cod muscle and effect of frozen storage. Ibid., **23**: 1025–1036.

COHEN, J., M. HAMOSH, R. ATIA, AND B. SHAPIRO. 1967. Lysolecithinase in fish muscle. J. Food Sci., **32**: 179–181.

DYER, W. J. 1967. Frozen fish muscle. Chemical changes and organoleptic quality. Cryobiology, **3**: 297–305.

DYER, W. J., AND M. L. MORTON. 1956. Storage of frozen plaice fillets. J. Fish. Res. Bd. Canada, **13**: 129–134.

FAGERLUND, U. H. M. 1967. Plasma corticol concentration in relation to stress in adult sockeye salmon during the fresh water stage of their life cycle. Gen. Comp. Endocrinol., **8**: 197–207.

GALLAI-HATCHARD, J. J., AND R. H. S. THOMPSON. 1965. Phospholipase A activity of mammalian tissues. Biochim. Biophys. Acta, **98**: 128–136.

HÖGBERG, B., AND B. UNVÄS. 1957. Release of histamines from mast cells by phospholipase A resulting in formation of lysolecithin. Acta Physiol. Scand., **41**: 345–369.

JONAS, R. E. E., AND E. BILINSKI. 1967a. Glycerylphosphorylcholine and related compounds in rainbow trout muscle stored at –4 C. J. Fish. Res. Bd. Canada, **24**: 273–280.

1967b. Phospholipase A activity in rainbow trout muscle. Ibid., **24**: 2555–2562.

KELLER, R. 1962. Nachweis von Lecithin bzw. Lysolecithin in Lipoid Extrakten normaler bzw. anaphylaktischer Mastzellen. Helv. Physiol. Acta, **20**: C 66.

KLIBANSKI, C., AND A. DE VRIES. 1964. Lysophospholipid breakdown in cobra venom-treated rabbit erythrocytes. Toxicon, **2**: 181–186.

LOVERN, J. A., AND J. OLLEY. 1962. Inhibition and promotion of postmortem lipid hydrolysis in the flesh of fish. J. Food Sci., **27**: 551–559.

LOVERN, J. A., J. OLLEY, AND H. WATSON. 1959. Changes in the lipids of cod during storage in ice. J. Sci. Food Agr., **10**: 327–337.

MacLeod, R. A., R. E. E. Jonas, and E. Onofrey. 1960. A biochemical study of coho salmon (*Oncorhynchus kisutch*) maturing sexually in an aquarium. J. Fish. Res. Bd. Canada, **17**: 323–335.

Marples, E. A., R. H. S. Thompson, and G. R. Webster. 1959. The liberation of active enzymes from brain tissue by lysolecithin. J. Neurochem., **4**: 62–70.

McArdle, B., R. H. S. Thompson, and G. R. Webster. 1960. The action of lysolecithin and of snake venom on whole-cell preparations of brain, muscle and liver. Ibid., **5**: 135–144.

Mellors, A., and A. L. Tappel. 1967. Hydrolysis of phospholipids by a lysosomal enzyme. J. Lipid Res., **8**: 479–485.

Olcott, H. H. 1967. Antioxidants. *In* M. E. Stansby [ed.] Fish oils. The Avi Publishing Company Inc., Westport, Connecticut. p. 164–170.

Olley, J., and J. A. Lovern. 1960. Phospholipid hydrolysis in cod flesh stored at various temperatures. J. Sci. Food Agr., **11**: 644–652.

Olley, J., R. Pirie, and H. Watson. 1962. Lipase and phospholipase activity of fish skeletal muscle and its relationship to protein denaturation. Ibid., **13**: 501–516.

Ottolenghi, A., and F. Bernheim. 1961. Lecithinase and lysolecithinase activity of rat intestinal mucosa after whole-body X-irradiation. Radiation Res., **15**: 609–615.

Steyermark, A. 1951. Quantitative organic microanalysis. Blackiston Co., Toronto, Ont. p. 134.

Tarr, H. L. A. 1941. Formation of drip in fish muscle and its control in defrosted fish. Fish. Res. Bd. Canada, Pacific Progr. Rept. No. 47. p. 5–9.

Thompson, R. H. S. 1964. Lipolytic enzymes and demyelination. *In* R. M. C. Dawson and D. N. Rhodes [ed.] Metabolism and physiological significance of lipids. John Wiley and Sons Ltd., London, New York, Sidney. p. 541–551.

Tomlinson, N., S. E. Geiger, and S. W. Roach. 1967. Quality of sea frozen fish from the north eastern Pacific ocean. F.A.O. Tech. Conf. on freezing and irradiation of fish. FE:FTI/67/0/1.

Waite, M., and L. L. M. van Deenen. 1967. Hydrolysis of phospholipids and glycerides by rat liver preparations. Biochim. Biophys. Acta, **137**: 498–517.

Yurkowski, M., and H. Brockerhoff. 1965. Lysolecithinase of cod muscle. J. Fish. Res. Bd. Canada, **22**: 643–652.

Smolt Survival and Adult Utilization of Little Codroy River, Newfoundland, Atlantic Salmon[1]

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ABSTRACT

Between 1954 and 1961, seaward-migrating Atlantic salmon smolts were marked by the removal of their dorsal and adipose fins and released from the counting fence in the estuary of the Little Codroy River, Newfoundland, to determine their survival to, and utilization as, adults. Not all smolts were marked: in 1957 and 1958, about half, and in 1959, none were marked to assess the effects of marking on survival; in 1962, smolts were counted without being handled in a specially designed trap to assess the effects of handling on survival. A total of 61,394 smolts were finclipped; 523 marked adults (0.82% of the smolts marked) returned to the fence, and 876 marked adults (1.47%) were estimated to have been taken by the commercial fishery in Newfoundland and Labrador. In addition, there was an unknown but probably large number of grilse landed that did not get into commercial channels and so were not sampled. Five age-groups of smolts migrated, but two of these were scarce and only three were found among the adult survivors. These were age-2 smolts (1.79% of the smolts marked), age-3 smolts (2.43%), and age-4 smolts (1.64%). Three sea-age groups were represented among the recorded survivors: grilse (0.96% of the smolts marked), 2 sea-year salmon (1.21%), and 3 sea-year salmon (0.07%). About 62% of the recorded survivors were utilized by the commercial fishery, 4% by anglers on the Little Codroy, and the remaining 34% escaped and may have spawned. The commercial fishery is estimated to have taken 48% of the grilse (plus some unrecorded grilse), 75% of the 2 sea-year salmon, and 43% of the 3 sea-year salmon. The handicap created by the loss of the dorsal and adipose fins and defined by the ratio of the instantaneous mortality rates of marked and unmarked fish was estimated to be 1.10. Handling smolts was estimated to cause an immediate instantaneous mortality of 0.23. The overall average survival rate, conservatively adjusted, becomes 3.64%, that is, 2235 adult salmon would have survived from the migration of 61,394 smolts if they had not been handled and marked. However, if an estimate of unrecorded grilse be added, and a greater adjustment made for marking mortality, the survival rate becomes 9.42%. In addition to handling and marking, smolt survival also appears to have been affected by the size, age, and number of smolts migrating, as well as by the mark itself. Returns of Little Codroy smolts to the fence were lower than returns of salmon with missing adipose and ventral fins reported from several other streams.

INTRODUCTION

The decline of commercial landings of Atlantic salmon (*Salmo salar* Linnaeus) in Canada and Newfoundland from a peak of 13.4 million lb in 1930 to 6.4 million lb in 1949 was considered serious enough in 1949 to warrant special efforts at recovery. A committee sponsored by the federal government and the governments of the five Atlantic provinces was formed to investigate the factors affecting production, to improve management, and to coordinate the

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efforts of the participating agencies (Kerswill, 1961). Topics of special importance were the annual production of smolts by certain rivers, survival at sea, and utilization of adults.

In 1952 a long-term investigation of Atlantic salmon was advancing on three rivers, the Port Daniel in the Gaspé region of Quebec, and the Pollett and Miramichi in New Brunswick (Fig. 1). A fourth, the Little Codroy, was

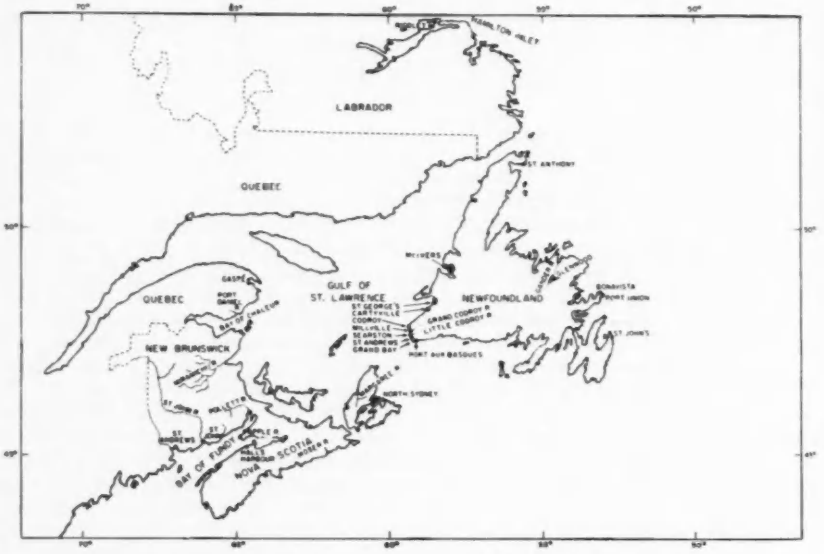

FIG. 1. Eastern Canada showing locations of places mentioned in the text.

added in 1953. The Little Codroy was selected because it had an adequate stock of salmon, it was small and accessible, and it flowed into the Gulf of St. Lawrence as did two other rivers of the experiment.

This paper summarizes the results of the Little Codroy investigations. Research there had two objectives: the first to estimate survival during the smolt–adult phase of the life cycle, and the second to assess the effect of the marking and handling techniques on survival.

EXPERIMENTAL PROCEDURES

The primary purpose, estimation of survival during the smolt–adult phase, was achieved by counting and distinctively finclipping the seaward migrants and by estimating the number of marked adults taken by the commercial fishery and the number that returned to the river. At a counting fence of netting, located in the estuary of the Little Codroy downstream from all tributaries and about 1.6 km from the sea, smolts were captured each spring and upstream migrating adults each summer and autumn from 1954 to 1963.

The second objective, assessment of the effect of handling and marking on smolt survival, was achieved by handling smolts in various ways at the counting fence, by marking smolts upstream, and by estimating the number of survivors subsequently taken at the fence. Murray (MS, 1968a) described the counting fence and traps. The approximate location of the fence and certain commercial nets where adults were sampled is shown in Fig. 2.

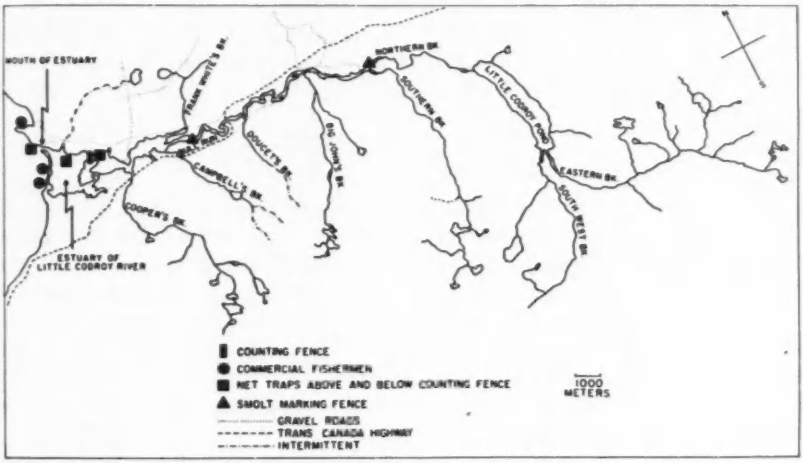

FIG. 2. Relative locations of counting fence and traps in the Little Codroy River and commercial and sampling nets in the sea near the mouth of the Little Codroy estuary.

AGE DETERMINATION AND DEFINITIONS

Ages were determined from scales taken from a small area on the left side of the body, above the lateral line and between the dorsal and adipose fins. A series of widely separated circuli (summer growth) and closely spaced circuli (winter growth) represented a year of growth. The outer margin of the winter growth was the point used to separate one year from another.

Little Codroy Atlantic salmon are anadromous and their scales exhibit freshwater and ocean growth. Smolt and sea ages are designated separately; the ages also indicate the number of growing seasons. A 2-year-old smolt, for example, has completed two growing seasons in fresh water and migrated to sea early in its 3rd year of life. Similarly, a 1 sea-year salmon has spent one growing season, and often part of a second, in the ocean; it may have spent 2, 3, or more years in fresh water before migrating to sea. A decimal notation is used herein to designate smolt and sea ages; age 2.1, for example, indicates that the smolt age is 2 years, or age 2., and the sea age is 1 year, or age .1.

Some adult Atlantic salmon are called grilse and others "salmon." *Grilse* have spent one growing season at sea and typically weigh less than 2.9 kg round. "*Salmon*" include all adults older and larger than grilse; the word will

always appear between quotation marks when used in this sense to distinguish it from the word salmon meaning Atlantic salmon.

Adult Atlantic salmon that have not spawned are called *maiden* salmon. Most adults do not die when they have spawned but return to sea during the winter or spring after spawning; the downstream migrants are often called *kelts*. Some previously spawned salmon are subsequently taken by the commercial fishery and some return to spawn again. Scales from such fish show areas of resorption or "spawning marks."

The investigations to be described revealed that Little Codroy smolts and adults encountered various kinds of mortality in the river and ocean. The kinds of mortality and where these occurred are shown schematically in Fig. 3.

Counting, Handling, and Marking Smolts

Smolts were counted and marked on a work stage set just downstream from the trap where smolts were caught (Murray, MS, 1968a). They were taken from the elevator of the trap with a small dipnet and groups of 30–40 were placed in a wooden tub containing about 45 liters of water from the estuary. Individuals were taken from the tub and the dorsal and adipose fins were removed with scissors at the level of the flesh. Each finclipped smolt was then placed in a recovery box floating beside the work stage; this box had wooden sides and ends but the bottom was covered with wire cloth so it remained partially submerged.

All of the smolts in five runs and about half in two runs of 1954–61 were marked by the removal of the dorsal and adipose fins (Table I). One man did the marking in each smolt run but not all runs were marked by the same man.

Considerable handling was involved in this work. The term "handling" as used herein, not only means the act of finclipping, but also means transferring smolts from the elevator of the trap to a tub of water and placing them in the recovery box. The last two procedures also involved crowding. When large numbers of fish were crowded in the elevator, it was kept out of the river just long enough to allow a man to scoop some smolts from it with a small dipnet. In addition, smolts were sometimes crowded in the tub and recovery box. Crowding in the elevator and tub was kept to a minimum by working as quickly as possible. Holding time in the recovery box depended on the number of smolts in the trap and whether they had been anaesthetized. Anaesthetized smolts were usually kept 3–6 hr before being released; smolts that had not been anaesthetized were released 10–60 min after marking.

A sample of smolts from each run was measured (fork and standard lengths), weighed, and scale-sampled. Sex was determined and stomach contents collected from all smolts measured in 1956 and later. In 1954 and 1955 every 10th smolt was sampled, in 1956 every 40th, in 1957 every 30th, and from 1958 to 1961 every 20th. The number of smolts of each age in each run was estimated from the proportion of each age in each sample.

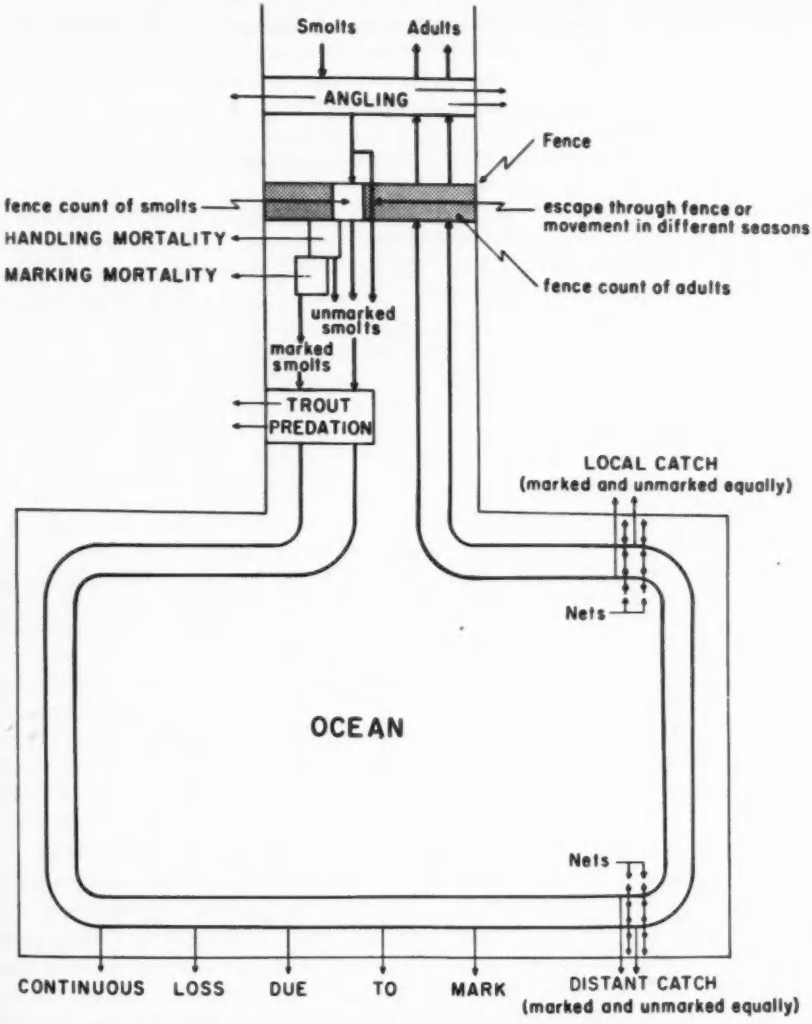

FIG. 3. Diagram showing the kinds of mortality encountered by Little Codroy Atlantic salmon smolts and adults and where these occurred.

TESTING THE EFFECT OF HANDLING AND MARKING ON SURVIVAL

The effects of handling and marking were evaluated by three tests at the counting fence in the estuary and by two tests in the river upstream. Tests at the fence involved anaesthetization, elimination of marking, and elimination of handling. Tests upstream from the fence consisted of marking smolts in various ways before and during their seaward migration.

TABLE I. Atlantic salmon smolts counted, marked, and released in the Little Codroy River, 1954–63.

Year	Procedure		Counted	Released		
	Handled	Anaesthetized		Finclipped	Unmarked	Total
1954	Yes	Yes	12,210	12,030	0	12,030
1955	"	"	11,248	11,154	0	11,154
1956	"	"	14,772	14,401	0	14,401
1957	"	No	8,900	4,450	4,157	8,607
1958	"	Yes	9,341	4,671	4,203	8,874
1959	"	No	12,099	0	11,473	11,473
1960	"	"	7,829	7,165	0	7,165
1961	"	"	8,058	7,523	0	7,523
1962	No	"	8,193	0	7,579	7,579
1963	"	"	7,326	0	6,960	6,960
Total			99,976	61,394	34,372	95,766

SMOLTS AT THE FENCE

To facilitate marking in 1954, 1955, 1956, and 1958, smolts were anaesthetized with urethane (ethyl carbamate) at a concentration of 5 g per liter of water (Table I). They were kept lightly anaesthetized by placing them in a tub of anaesthetic for a short time and then in a tub of water; a basket of nylon bobbinet was used to hold smolts in the tubs and during transfer.

Every other smolt in 1957 and 1958 was finclipped at the fence (Table I). More finclipped smolts than nonfinclipped were released; fish killed for the length–weight–sex samples were selected from those which were not being finclipped.

None of the smolts in 1959 were finclipped (Table I, see also Fig. 3). Smolts handled but not finclipped were treated like smolts that were handled and finclipped, that is, they were taken from the tub individually by hand and held momentarily before being placed in the recovery box. These tests were evaluated by the number of adults subsequently recorded at the counting fence.

Smolts in the runs of 1962 and 1963 were counted without being handled (Table I, see also Fig. 3). Elimination of handling involved a different smolt trap (Murray, MS, 1968a). This trap was larger than the one used previously, and the elevator was divided into two compartments. The upstream compartment was a basket with a door at its downstream end; the downstream compartment contained a small plexiglass box mounted behind the door.

Smolts were guided with a seine into the upstream compartment of the elevator from the trap. The gate separating the trap and elevator pound was then closed, and the elevator raised to within about 15 cm of the river surface and held there. The door to the plexiglass box was then opened and

a few smolts admitted from the upstream compartment. One man with a hand tally counted these, and released them through a door at the downstream end of the box. More smolts were then admitted to the box, and the procedure repeated until all smolts in the upstream compartment had been counted. Every 20th smolt was removed from the plexiglass box with a small dipnet and data taken as described previously.

SMOLTS MARKED UPSTREAM FROM THE FENCE BEFORE MIGRATION

Smolt-sized parr, captured by electrofishing (Murray, 1958) during April and May in the tributary streams of the Little Codroy (Fig. 2), provided fish for some of the tests upstream from the fence (Table II). Collections were small in 1957, 1958, and 1959, but in 1960 and 1961, about 1.6 km of each tributary were systematically fished. All sizes of young salmon were collected. The silvery coat of guanine was not apparent on all fish large enough to be smolts. All salmon that were either silvery or that were longer than about 14 cm fork length were marked because most smolts were longer than this (Table III).

The man doing the finclipping at the main fence examined each smolt for marks before removing any fins (or before placing it in the recovery box if it was not to be clipped). Smolts marked with internal anchor tags were easily recognized by the feel of the protruding wire. Each tagged smolt was killed and data taken as described previously.

SMOLTS MARKED UPSTREAM FROM THE FENCE DURING MIGRATION

Migrating smolts captured upstream from the main fence in a trap of netting (Murray, MS, 1968a) in 1960 and 1961, and at a scaled-down version of the fence in 1961 and 1962, provided smolts for the remaining tests upstream from the fence (Table II). The trap of netting was set about 400 m upstream from the main fence (Fig. 2) and the dorsal fin was removed from every smolt captured in it.

The smolt trap in the fence used upstream was similar to that used in the main fence in the estuary between 1954 and 1960, except that it was smaller (3 m square and 1 m high). Nets were suspended from wire ropes stretched upstream and diagonally across the river from the trap to form a V-shaped entrance to it. In 1961, this fence was set about 4 km upstream from the estuarial fence, and in 1962, it was set in Northern Brook about 13 km upstream (Fig. 2 and Table II). Smolts were handled as at the main fence. The dorsal and adipose fins were removed from every smolt captured in the trap.

In 1960 and 1961, finclipped smolts were found by the man doing the marking at the main fence. In 1962, they were observed by the fresh scars of the missing dorsal fins as the smolts entered the plexiglass box in the elevator of the smolt trap. All finclipped smolts were killed and data taken as described previously.

Sampling of Adults

Adults bearing Little Codroy marks were taken by the commercial salmon fishery in Newfoundland and Labrador (Fig. 3). Special observers were em-

TABLE II. Atlantic salmon smolts marked and recaptured, as smolts, in the Little Codroy River, 1957–62.

Summary of marking information					No. recaptured			
Year	Method of capture	Distance upstream from fence (*km*)	Mark applied	No. marked	Fence	Trap 400 m upstream from fence	Angling	Total
1957	Electrofishing	2.2–11.7	Loop of cotton thread just anterior to dorsal fin	223	14	–	–	14
1958	"	2.2–13.4	Loop of 0.127-mm diam nickel wire just anterior to dorsal fin	40	14	–	–	14
1959	"	2.2–14.3	Loop of 0.127-mm diam nickel wire just anterior to dorsal fin	57	14	–	–	14
1959	"	2.2–14.3	Internal anchor tag[a]	80	22	–	–	22
1960	"	1.0–14.3	Internal anchor tag[a]	931	295	61	128	484
1960	Net trap	0.4	Dorsal fin removed	797	460	–	–	460
1961	Electrofishing	1.0–14.3	Internal anchor tag[a]	533	138	44	55	237
1961	Net trap	0.4	Dorsal fin removed	1179	796	–	–	796
1961	Small duplicate of fence	4.0	Dorsal and adipose fins removed	14	4	–	–	4
1962	Small duplicate of fence	12.9	Dorsal and adipose fins removed	405	236	–	–	236
Total			Thread loop	223	14	–	–	14
			Wire loop	97	28	–	–	28
			Internal anchor tag[a]	1544	455	105	183	743
			Finclipping	2395	1496	–	–	1496
			Total	4259	1993	105	183	2281

[a]Internal anchor tags were celluloid, 13 × 3 × 0.5 mm thick. Each tag contained a number printed with black vinyl ink; nickel wire, 0.254 mm in diam and 16 mm long, was fastened to one end.

TABLE III. The numbers of Little Codroy River Atlantic salmon smolts finclipped and released from the counting fence, 1954–61, and finclipped adults recaptured, 1955–63.

Smolt run	Smolt age	Estimated no. of each age finclipped and released	Avg fork length (*mm*)	No. of finclipped adults of each smolt age and sea age recaptured																					
				Commercial fishery																					
				In Newfoundland and Labrador					In Maritimes	Near mouth Little Codroy River estuary					Angling Gander River	Counting fence Little Codroy River					Total returns				
				.1[a]	.2	.3	PS[b]	T[c]	.2	.1	.2	.3	PS	T	.1	.1	.2	.3	PS	T	.1	.2	.3	PS	T
1954	2.	5,185	149	–	6	–	–	6	–	14	13	2	1	30	–	15	9	2	–	26	29	28	4	1	62
	3.	6,749	155	–	2	–	–	2	–	19	25	–	–	44	–	71	20	–	–	91	90	47	–	–	137
	4.	96	158	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	?	–	–	1	–	–	–	1	1	1	1	–	–	2	–	2	–	–	–	2	4	2	–	–	6
	T[c]	12,030	152	1	8	–	–	9	1	34	39	2	1	76	–	88	29	2	–	119	123	77	4	1	205
1955	2.	4,573	145	2	6	1	–	9	–	8	6	–	1	15	–	19	12	3	1	35	29	24	4	2	59
	3.	6,447	158	3	17	–	–	20	–	23	10	–	–	33	–	37	20	1	–	58	63	47	1	–	111
	4.	134	170	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	?	–	–	–	–	–	–	–	1	2	–	–	–	2	–	3	2	2	–	7	5	3	2	–	10
	T	11,154	153	5	23	1	–	29	1	33	16	–	1	50	–	59	34	6	1	100	97	74	7	2	180
1956	2.	5,184	144	3	28	2	–	33	–	16	18	1	1	36	–	18	16	2	–	36	37	62	5	1	105
	3.	8,785	152	9	52	1	1	63	–	24	29	–	4	57	–	60	29	–	1	90	93	110	1	6	210
	4.	389	152	–	3	–	–	3	–	–	2	–	–	2	–	1	–	–	–	1	1	5	–	–	6
	5.	43	128	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	?	–	–	–	3	–	–	3	–	4	5	2	1	12	3	5	–	–	–	5	12	8	2	1	23
	T	14,401	148	12	86	3	1	102	–	44	54	3	6	107	3	84	45	2	1	132	143	185	8	8	344
1957	2.	1,175	132	–	10	1	–	11	–	1	2	1	–	4	–	3	6	2	–	11	4	18	4	–	26
	3.	3,245	144	6	31	–	–	37	–	12	12	1	1	26	–	22	8	6	–	36	40	51	7	1	99
	4.	30	143	–	1	–	–	1	–	–	–	–	–	–	–	–	–	–	–	–	–	1	–	–	1
	?	–	–	–	–	–	–	–	–	2	1	–	–	3	–	–	–	–	–	–	2	1	–	–	3
	T	4,450	140	6	42	1	–	49	–	15	15	2	1	33	–	25	14	8	–	47	46	71	11	1	129

(*Continued*)

TABLE III. The numbers of Little Codroy River Atlantic salmon smolts finclipped and released from the counting fence, 1954–61, and finclipped adults recaptured, 1955–63. — (*Concluded*)

Smolt run	Smolt age	Estimated no. of each age finclipped and released	Avg fork length (*mm*)	No. of finclipped adults of each smolt age and sea age recaptured																					
				Commercial fishery																					
				In Newfoundland and Labrador					In Maritimes	Near mouth Little Codroy River estuary					Angling Gander River	Counting fence Little Codroy River					Total returns				
				.1[a]	.2	.3	PS[b]	T[c]	.2	.1	.2	.3	PS	T	.1	.1	.2	.3	PS	T	.1	.2	.3	PS	T
1958	2.	1,364	141	–	–	2	–	2	–	1	4	1	–	6	–	4	1	–	–	5	5	5	3	–	13
	3.	3,130	152	6	2	–	–	8	–	8	9	–	1	18	–	12	4	3	–	19	26	15	3	1	45
	4.	168	168	–	–	–	–	–	–	1	–	–	–	1	–	2	–	–	–	2	3	–	–	–	3
	6.	9	195	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	?	–	–	–	–	–	–	–	–	–	1	–	–	1	–	1	–	–	–	1	1	1	–	–	2
	T	4,671	149	6	2	2	–	10	–	10	14	1	1	26	–	19	5	3	–	27	35	21	6	1	63
1960	2.	1,677	143	3	–	1	2	6	–	3	–	–	–	3	–	2	6	2	1	11	8	6	3	3	20
	3.	5,338	151	9	15	–	1	25	–	22	–	–	–	22	–	10	14	3	1	28	41	29	3	2	75
	4.	150	158	–	–	–	–	–	–	1	–	–	–	1	–	1	1	–	–	2	2	1	–	–	3
	?	–	–	1	–	–	–	1	–	1	–	–	–	1	–	1	–	–	–	1	3	–	–	–	3
	T	7,165	149	13	15	1	3	32	–	27	–	–	–	27	–	14	21	5	2	42	54	36	6	5	101
1961	2.	4,228	140	–	24	–	–	24	–	–	–	–	–	–	–	17	11	–	–	28	17	35	–	–	52
	3.	2,919	150	–	14	–	–	14	–	–	–	–	–	–	–	11	14	–	–	25	11	28	–	–	39
	4.	376	154	–	–	–	–	–	–	–	–	–	–	–	–	–	1	–	–	1	–	1	–	–	1
	?	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	2	–	–	2	–	2	–	–	2
	T	7,523	144	–	38	–	–	38	–	–	–	–	–	–	–	28	28	–	–	56	28	66	–	–	94
Totals	2.	23,386	144	8	74	7	2	91	–	43	43	5	3	94	–	78	61	11	2	152	129	178	23	7	337
	3.	36,613	153	33	133	1	2	169	–	108	85	1	6	200	–	223	109	13	2	347	364	327	15	10	716
	4.	1,343	160	–	4	–	–	4	–	2	2	–	–	4	–	4	2	–	–	6	6	8	–	–	14
	5.	43	128	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	6.	9	195	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	?	–	–	2	3	–	–	5	2	10	8	2	1	21	3	12	4	2	–	18	27	17	4	1	49
	T	61,394	150	43	214	8	4	269	2	163	138	8	10	319	3	317	176	26	4	523	526	530	42	18	1,116

[a]Sea age.
[b]Previously spawned.
[c]Total.

ployed each summer from 1953 to 1963 to search for salmon with missing fins among the commercial landings from Newfoundland. One was stationed at North Sydney, N.S., each year; another at Saint John, N.B., from 1953 to 1961; and another at Bonavista, Nfld., from 1960 to 1963 (Fig. 1). The Newfoundland observer also worked at Port Union in 1960 and 1961, and at St. Anthony in 1962 and 1963. The numbers of salmon examined were recorded and finclipped salmon were weighed and scale-sampled. The description of the work of the observers, the number of salmon examined, and the number of salmon found with missing fins, are given in Murray (MS, 1966).

In addition to the work of the special observers, staff employed on the Little Codroy project collected information from the two (occasionally three) men who fished commercially near the mouth of the estuary during June and July each year from 1954 to 1961 (Fig. 2). All the finclipped salmon caught by these men, and many unmarked ones, were measured, weighed, and scale-sampled; in addition, the sex of many was obtained. It was not possible to see all the salmon landed, but the total numbers caught were reported.

Each summer and autumn from 1954 to 1963, adult salmon migrating upstream in the Little Codroy River were captured at the same counting fence in the estuary where smolts were captured (Fig. 3). Individual adults were anaesthetized with urethane, at a concentration of 5 g per liter of water, from 1954 to 1958, and tricaine methanesulfonate (M.S. 222) at a concentration of 0.05 g per liter of water, from 1959 until 1963. While anaesthetized, each adult salmon was examined for missing fins, measured (fork and standard lengths), scale-sampled, and tagged with an Atkins-type tag (Rounsefell and Kask, 1945). In 1954, the tags were of dark red vinylite, 31.8 × 7.9 × 0.8 mm thick, and were attached just anterior to the dorsal fin with nickel wire 0.6 mm in diam and 7.6 cm long. They were easily seen in shallow water even when the fish were inconspicuous, so in 1955 and later years, the tags were of transparent cellulose nitrate, 33.3 × 7.9 × 0.8 mm thick, and were attached to the fish with the same type and length of nickel wire described above. The method of attaching the tags is described by Blair (1956). After each salmon was tagged, it was placed in a bag of nylon bobbinet, weighed on a spring balance, and placed in a recovery box. Most salmon recovered within a few minutes and were released just upstream from the fence a short time after that.

Seaward migrating kelts were captured at the counting fence along with the smolts each spring from 1954 to 1963. The method of handling these fish in the smolt trap is described in Murray (MS, 1968a). Each kelt was measured, weighed, scale-sampled, and examined to see if it was tagged or finclipped. Untagged kelts were assumed to have entered the river after the fence had been removed in the autumn; they were marked with the Atkins-type tags described above.

Tagged salmon released upstream from the fence were available to anglers (Fig. 3), and the return of tags was used to provide a measure of utilization

[b]Previously spawned.
[c]Total.

by angling in the Little Codroy River. Anglers not resident in the area usually employed guides, and periodically one of the crew visited these men to collect tags and capture information. A reward of $1.00 was given for the return of each tag.

Guardians and fishery officers of the Department of Fisheries of Canada see many of the salmon caught by anglers fishing Newfoundland rivers each year. These men were asked to look for salmon with missing fins in the angling catches. During the period 1953–63, observations of salmon caught by anglers on some rivers were sent to the Biological Station, St. Andrews, N.B.

DEFICIENCIES IN THE BASIC DATA

The basic data are summarized in Table III. A total of 61,394 smolts was finclipped and released from the Little Codroy counting fence, and 1116 finclipped adults were recovered. The returns are divided into five categories: counting fence, commercial nets near the mouth of the Little Codroy estuary, commercial fishery elsewhere in Newfoundland and Labrador, commercial fishery in the Maritimes, and angling. The latter does not include angling in the Little Codroy because anglers there took salmon that had already been recorded at the fence, and these data are summarized in Table IV. The three fish in the angling category in Table III were reported by fishery officers of the Department of Fisheries of Canada to have been angled on Gander River in 1956 (Fig. 4); two were caught near the mouth of the river and the third was caught at Glenwood about 43 km upstream from the mouth.

Deficiencies in Counting Fence Data

The Little Codroy counting fence data contain three major deficiencies. First, the 523 finclipped adults recorded between 1955 and 1963 (Tables III and IV) may not include all the marked salmon that returned to the river. Some survivors may have been turned away by the fence in the estuary and returned to sea; others may have entered the river after the fence had been removed in the autumn and returned to sea before it was set out in the spring.

There is some evidence for these inferences. Fifty-five tagged adults were released downstream from the fence during the summers 1958–63. Thirty-two were recaptured at the fence during the summer of tagging after an average interval of 18 days, and two were recaptured as kelts at the fence in the spring after tagging, 309 days later. The remaining 21 adults were not recaptured.

Similar results were obtained when 18 adults were tagged and released from a trap of netting 800 m downstream from the fence (Fig. 2) during the summers of 1961 and 1962. Nine were recaptured at the fence during the year of tagging after an average interval of 17 days. Two were recaptured at the fence during the year after tagging, one as a kelt in the spring 286 days later, and the other 364 days later in one of the upstream traps. The scales of the latter indicated that it had spawned during the previous year. Two others were recaptured at the trap from which they were released about 20

TABLE IV. The numbers of Little Codroy Atlantic salmon smolts finclipped and released from the counting fence, 1954–61, and finclipped adults recaptured at the fence, by anglers on the Little Codroy, and the escapement, 1955–63.

Smolt run	No. of finclipped smolts released	Year adults recaptured	Sea age	No. of finclipped adults		
				Recorded at counting fence	Taken by anglers	Escapement
1954	12,030	1955	.1	88	7	81
		1956	.2	29	2	27
		1957	.3	2	–	2
			Total	119	9	110
1955	11,154	1956	.1	59	8	51
		1957	.2	34	5	29
		1958	.3	6	–	6
		1957	PS[a]	1	–	1
			Total	100	13	87
1956	14,401	1957	.1	84	9	75
		1958	.2	45	3	42
		1959	.3	2	–	2
		1959	PS[a]	1	–	1
			Total	132	12	120
1957	4,450	1958	.1	25	–	25
		1959	.2	14	1	13
		1960	.3	8	–	8
			Total	47	1	46
1958	4,671	1959	.1	19	2	17
		1960	.2	5	–	5
		1961	.3	3	–	3
			Total	27	2	25
1960	7,165	1961	.1	14	–	14
		1962	.2	21	1	20
		1963	.3	5	–	5
		1962	PS[a]	2	–	2
			Total	42	1	41
1961	7,523	1962	.1	28	–	28
		1963	.2	28	8	20
			Total	56	8	48
Total	61,394		.1	317	26	291
			.2	176	20	156
			.3	26	–	26
			PS[a]	4	–	4
			Total	523	46	477

[a]Previously spawned.

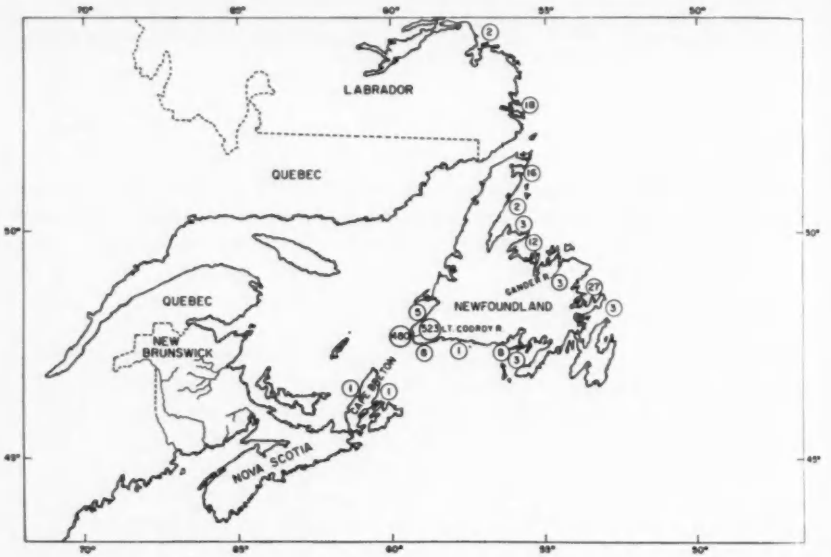

FIG. 4. Recaptures of adult Atlantic salmon with dorsal and adipose fins missing. The numbers of fish are given inside the circles.

days after they were marked; they were released again but they were not recaptured at the fence. The remaining five fish were not recaptured.

Adults with no missing fins were observed at the counting fence (Table V) even when none were expected (i.e., when all smolts in a run had been marked). These fish are a second deficiency in the data: some may have been survivors of smolts that escaped marking; others may have strayed into the Little Codroy from other rivers. The numbers of unmarked adults observed at the fence in the years after 1954, 1955, 1956, 1960, and 1961 (Table V) are approximately proportional to the total number of adults recorded there (Fig. 5). If the unmarked adults were strays from other rivers, their numbers would be independent of the number of finclipped adults. (The Grand Codroy, a large river just north of the Little Codroy is a likely source of stray salmon. I estimate that 2000–4000 adults enter the Grand Codroy annually, whereas only about 140 entered the Little Codroy each year.) If, on the other hand, the unmarked adults were a mixture of native and stray fish, the line fitting the points in Fig. 5 should have a positive intercept on the ordinate. Actually the best line fitted to the five points has a small negative intercept. There is therefore no evidence that any stray adults entered the Little Codroy; more conservatively, their numbers were negligible.

The nets of the "down" or smolt fence are believed to have been effective barriers to migrating smolts, and the counts reported in Table I are believed reasonably complete. The effectiveness of the fence was tested in 1960 and

TABLE V. The number and sea ages of finclipped and nonfinclipped maiden Atlantic salmon adults recorded at the Little Codroy counting fence, 1955–63.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
		No. of smolts			No. of maiden adults recorded at counting fence											
			Not marked		Finclipped				Nonfinclipped				Total			
Row no.	Smolt run	Marked	Handled	Not handled	.1[a]	.2	.3	Total	.1	.2	.3	Total	.1	.2	.3	Total
1	1954	12,030	–	–	88	29	2	119	10	6	1	17	98	35	3	136
2	1955	11,154	–	–	59	34	6	99	20	9	3	32	79	43	9	131
3	1956	14,401	–	–	84	45	2	131	34	11	3	48	118	56	5	179
4	1960	7,165	–	–	14	21	5	40	14	3	0	17	28	24	5	57
5	1961	7,523	–	–	28	28	3(est.)	59±	13	5	1(est.)	19±	41	33	4(est.)	78±
6	Total	52,273	–	–	273	157	18±	448±	91	34	8±	133±	364	191	26±	581±
7	Mean	–	–	–	54.6	31.4	5.6	89.6	18.2	6.8	1.6	26.6	72.8	38.2	5.2	116.2
8	1957	4,450	4,157	–	25	14	8	47	55	29	4	88	80	43	12	135
9	1958	4,671	4,203	–	19	5	3	27	34	17	5	56	53	22	8	83
10	Total	9,121	8,360	–	44	19	11	74	89	46	9	144	133	65	20	218
11	1959	–	11,473	–	0	0	0	0	45	23	12	80	45	23	12	80
12	1962	–	–	7,579	0	[b]	[b]	0	117	[b]	[b]	117	117	[b]	[b]	117
13	Total	–	11,473	7,579	0	[b]	[b]	[b]	162	[b]	[b]	197	162	[b]	[b]	197
14	Grand total	61,394	19,833	7,579	317	176	29±	522±	342	103	29±	474±	659	279	58±	996±

[a]Sea age.
[b]Not determined.

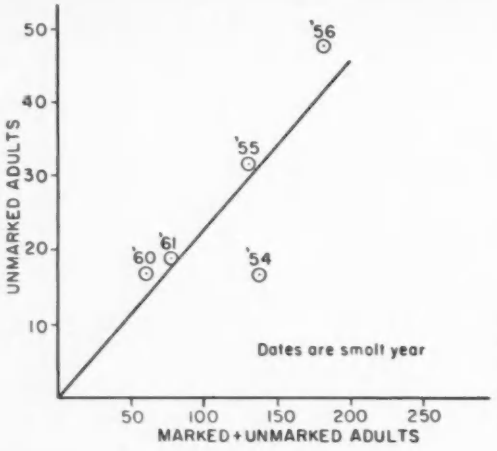

FIG. 5. Relationship between the number of marked and unmarked Atlantic salmon adults observed at the Little Codroy counting fence, 1955–61.

1961 when smolts captured in a trap of netting 800 m downstream from the fence (Fig. 2) were examined to see if they were finclipped (Table VI). The dorsal fin was clipped from each smolt captured at a trap of netting 400 m upstream from the fence, and the dorsal and adipose fins were clipped from every smolt at the fence. All 879 smolts captured at the downstream trap in 1960, and 465 of the 539 captured in it in 1961, had both the dorsal and adipose fins missing. No smolts with the dorsal fin missing were captured at the downstream trap. On the other hand, only 64% of those that were marked at the upstream trap were captured at the fence. The low recapture rate is believed to be the result of mortality from handling and marking rather than escapes through the fence. Some did escape however. Seventy-four unmarked smolts were captured in the downstream trap shortly after some nets in the fence were damaged in a flood in May 1961. After the fence was repaired, no more unmarked smolts were captured. It is believed that few smolts escaped through damaged nets in the fence because it was inoperative on three occasions only for a total of 7 days during 10 seasons and 1653 days of operation. Nevertheless unmarked native adults were observed at the fence in a number proportional to the number of marked adults (Fig. 5); these fish must be the survivors of smolts that migrated before the fence went in or that passed undetected through it.

The third deficiency in the counting fence data concerns the survival of young salmon marked before the smolt migration began (Table II). All young salmon that were either silvery or longer than 14 cm fork length were marked; it is possible that some parr were marked along with the smolts and did not migrate.

DEFICIENCIES IN COMMERCIAL FISHERY DATA

Fishery officers of the Department of Fisheries of Canada reported six salmon with missing dorsal and adipose fins from some commercial catches

TABLE VI. The number of finclipped and nonfinclipped Atlantic salmon smolts captured at the Little Codroy counting fence, at a trap 400 m upstream from it, and at a trap 800 m downstream from it, 1960 and 1961.

	Upstream trap		Main counting fence			Downstream trap		
	Captured	Marked and released	Captured	Recaptured	Marked and released	Captured	Recaptured	
Year	No missing fins	Dorsal fin clipped	No missing fins	Dorsal fin missing	Dorsal and adipose fins clipped	No missing fins	Dorsal fin missing	Dorsal and adipose fins missing
1960	797	797	7,369	460	7,165	0	0	879
1961	1,179	1,179	7,262	796	7,523	74	0	465
Total	1,976	1,976	14,631	1,256	14,688	74	0	1,344

in Nova Scotia. Two "salmon" were reported from Cape Breton, one in 1956 and the other in 1957 (Fig. 4), and four grilse were reported from Halls Harbour in the Bay of Fundy (Fig. 1), one in 1955 and three in 1957.

Between 1950 and 1958, 84,631 hatchery-reared Atlantic salmon parr were marked by the removal of the dorsal and adipose fins and planted in six Maine rivers (State of Maine Atlantic Sea Run Salmon Commission, 1952, 1960). Data on the survival of the planted fish are lacking but it is possible that the four marked grilse reported from the Bay of Fundy were marked in Maine and they have not been included in the data in Table III.

A total of 419 salmon with missing dorsal and adipose fins were reported by the special observers in the commercial landings from Newfoundland and Labrador (Murray, MS, 1966). Only 269 of these are included in Table III; their distribution is shown in Fig. 4. The remainder were excluded, some because they had been measured twice, and others because they had been improperly weighed and scale-sampled. The observers in North Sydney reported 91 finclipped salmon from St. Andrews, Newfoundland. These fish were among the ones caught near the mouth of the Little Codroy estuary; the records were not used because they had already been examined (Table III). The sea ages determined from the scales of the remaining 59 samples did not agree with that expected from the weight data recorded on the scale envelopes and the samples were discarded. The sea age of an adult salmon can usually be accurately predicted from its weight: on the average, age-.1 salmon weigh 1.5 kg; age-.2 salmon weigh 3.7 kg; and age-.3 salmon weigh more than 5.6 kg. The discrepancies in the samples were usually obvious, for example, the scales would be that of an age-.1 salmon but the weight would be that of an age-.3 salmon. Some of these samples also contained scales from more than one salmon. Even though the basic data from the commercial fishery contained, and may still contain errors, the records were retained to estimate utilization of salmon by the commercial fishery. This estimate can, at best, only be a rough approximation because it is based upon another assumption, the estimate of the number landed, the accuracy of which cannot be assessed.

SMOLT PREDATION BY TROUT AND COD

Methods

The peak of the Little Codroy smolt run coincided with spawning of capelin, *Mallotus villosus* (Müller), on the beaches of the long sand bar which protects the estuary from the sea (Fig. 2). Cod, *Gadus morhua* Linnaeus, were usually abundant near the mouth of the river at such times.

Two attempts at assessing predation of smolts by cod were made. In 1957, stomach contents of cod which were caught during June and July in a salmon trap near the mouth of the Little Codroy and in cod traps near Codroy and Grand Bay (Fig. 1) were examined. In 1959, about 550 m of linetrawl were set in the sea 244 m west-northwest from the mouth of the estuary. This gear was set about mid-June and it was lifted and examined daily for about 2 weeks. The stomach contents of cod captured by it were examined for smolts.

Stomachs of cod captured in one of the commercial salmon traps near the river were also available for examination during June and July.

Results

No smolts were found in the stomach contents of 1693 cod caught near Codroy and Grand Bay (Fig. 1) in 1957, and in the stomachs of 495 cod caught near the mouth of the Little Codroy River in 1959.

Sea trout, *Salvelinus fontinalis* (Mitchill), about 35 cm long and about 0.4 kg in weight, were occasionally observed attacking smolts shortly after they had been released from the recovery box at the counting fence (Fig. 3). Trout migrated to sea each spring at the same time as the smolts and some of them preyed upon the smolts. The amount of predation was not assessed, but it could be substantial because the trout were rather abundant, especially in the mouth of the estuary.

ESTIMATED NUMBERS OF SALMON IN COMMERCIAL LANDINGS

Estimating Procedure

Atlantic salmon are fished commercially at many places around the island of Newfoundland and as far north as Hamilton Inlet on the Labrador coast (Fig. 1 and 3). Most fishermen fish near shore using stationary gillnets, but traps are used by some men in some areas, and some drift netting occurs offshore near Port aux Basques. The total weight landed in each of the 15 statistical areas of the province (Fig. 6) is compiled by the Department of Fisheries of Canada. Numbers landed are not compiled; there are some figures available (Table VII) but they only represent the landings from five different places at different times.

Commercial salmon landings in Newfoundland and Labrador include grilse and "salmon." Calculation of the number landed depends upon the proportion of grilse present and the average weights of the two major size-classes. Mr C. R. Molson (1960, personal communication), an economist with the Department of Fisheries of Canada, St. John's, Nfld., estimated the grilse proportion landed in each statistical area of Newfoundland and Labrador for 1959. He stated that the figures were obtained from many sources, some of which were different for the same areas. Considerable personal judgement was used and he stressed that the proportions derived were rough approximations. I have applied Molson's proportions, despite their limitations, in the absence of more complete information to estimate the numbers of grilse and "salmon" landed in each area for the period 1955–63. The average weights of grilse (1.5 kg) and "salmon" (3.7 kg) were derived from data collected by the special observers in Newfoundland (Murray, MS, 1966).

Results

The total weight landed in each of the 15 statistical areas of Newfoundland and Labrador (Fig. 6) for the period 1955–63 is given in Table VIII. The

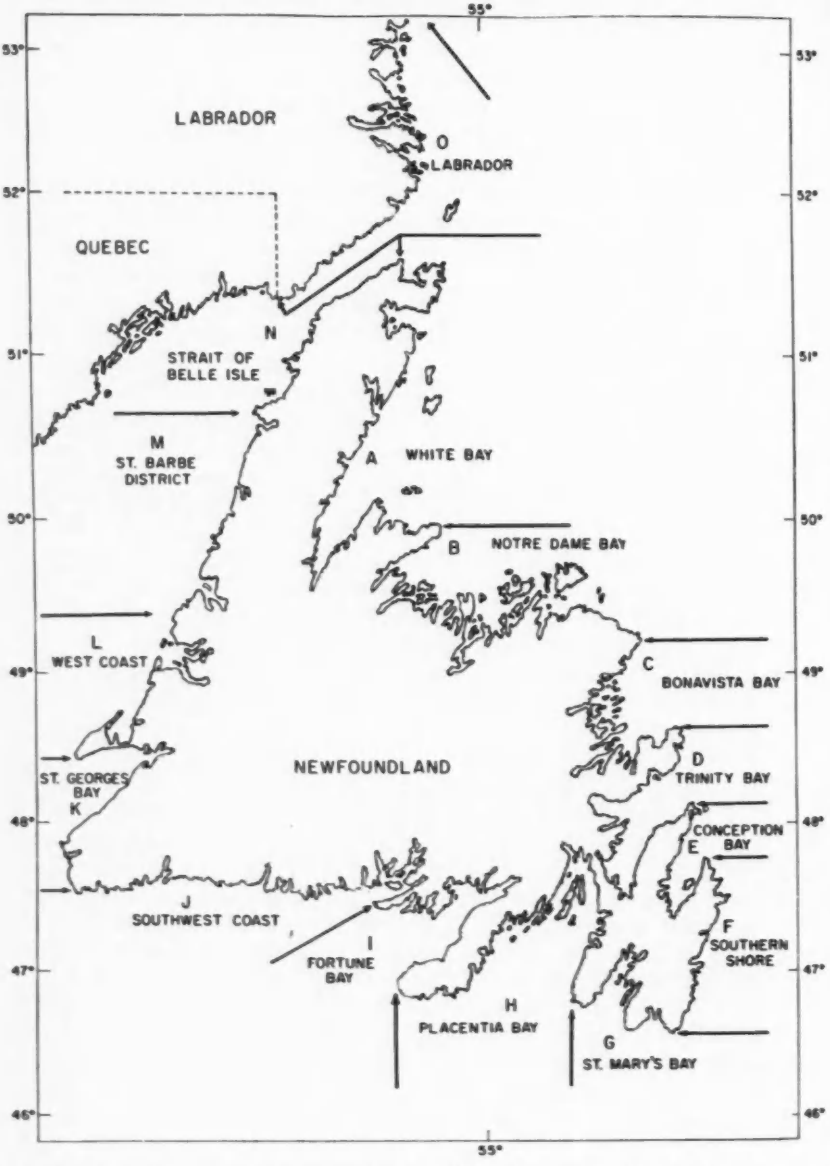

FIG. 6. The statistical areas of Newfoundland and Labrador as established by the Department of Fisheries of Canada.

TABLE VII. The numbers and proportion of Atlantic salmon grilse and "salmon" landed by the commercial fishery at five places in Newfoundland, 1942–63.

Place:	Bonavista	St. Andrews	Cartyville	St. George's	McIvers
Statistical area:	C	K	K	K	L
Year:	1960–63	1955–61	1943	1943	1942
Reference	Murray, MS, 1966	*	Blair, MS, 1966	Blair, MS, 1966	Blair, 1965
Grilse: No.	11,161	632	58	51	1,043
%	52	30	23	9	48
"Salmon": No.	10,488	1,473	191	492	1,148
%	48	70	77	91	52
Total no.	21,649	2,105	249	543	2,191

*Based on data collected during the course of the research reported in this paper.

TABLE VIII. The commercial landings of Atlantic salmon, in metric tons, round state, for the statistical areas of Newfoundland and Labrador, 1955–63. Data courtesy Department of Fisheries of Canada.

Area	1955	1956	1957	1958	1959	1960	1961	1962	1963
A	182.4	181.2	190.3	177.7	169.2	210.2	153.8	225.4	231.9
B	78.2	31.5	99.0	79.3	97.6	93.7	69.8	100.8	93.5
C	64.3	33.2	46.8	99.6	76.4	75.8	27.1	56.3	113.0
D	34.5	39.8	28.9	69.4	62.6	61.4	20.6	31.9	36.8
E	7.0	7.4	10.3	13.8	22.7	38.2	18.7	23.9	59.9
F	9.3	8.6	14.5	41.4	46.8	34.4	16.1	32.7	75.3
G	7.0	2.1	18.0	3.1	11.0	12.2	9.3	9.5	13.2
H	5.4	6.6	12.6	6.2	5.0	11.7	14.2	11.8	22.5
I	6.6	5.1	13.6	6.3	15.7	20.9	17.1	21.2	36.8
J	62.2	40.1	78.4	50.0	114.8	51.8	62.2	104.6	75.1
K	38.8	33.0	39.4	56.3	47.9	48.6	49.4	37.5	44.1
L	6.0	19.4	3.6	28.0	26.1	25.3	13.1	17.1	57.7
M	6.9	8.8	3.9	13.1	17.5	14.6	4.4	4.5	8.7
N	20.0	26.4	17.3	27.2	21.7	18.8	23.9	4.4	8.6
O	265.9	302.9	314.1	305.2	327.9	229.8	448.9	333.4	337.3
Total	794.5	746.1	890.7	976.6	1062.9	947.4	948.6	1015.0	1214.4

estimated numbers landed by area and year, and the estimated grilse proportion in each area for 1959 are given in Table IX.

ESTIMATED NUMBERS OF FINCLIPPED LITTLE CODROY SALMON TAKEN BY THE COMMERCIAL FISHERY

ESTIMATING PROCEDURES

Only a portion of the total commercial landings from Newfoundland and Labrador were examined by special observers. To estimate the number of

TABLE IX. The estimated numbers of Atlantic salmon grilse and "salmon" landed in each statistical area of Newfoundland and Labrador, 1955–63, based upon C. R. Molson's estimates of grilse proportions for each area in 1959.

Area	Estimated proportion (%)	Estimated no.								
		1955	1956	1957	1958	1959	1960	1961	1962	1963
					Grilse					
A	70	48,270	47,948	50,358	47,012	44,772	55,619	40,689	59,642	61,353
B	60	16,385	6,598	20,727	16,608	20,433	19,631	14,630	21,102	19,588
C	50	10,283	5,304	7,489	15,928	12,218	12,116	4,334	9,000	18,059
D	30	2,856	3,292	2,389	5,738	5,173	5,080	1,700	2,634	3,040
E	35	690	732	1,026	1,365	2,255	3,789	1,856	2,374	5,940
F	35	919	856	1,437	4,104	4,639	3,410	1,598	3,242	7,468
G	75	2,135	635	5,455	929	3,321	3,688	2,825	2,868	3,991
H	75	1,632	2,006	3,812	1,896	1,504	3,546	4,293	3,575	6,811
I	50	1,062	812	2,182	1,005	2,505	3,341	2,728	3,393	5,881
J	30	5,146	3,318	6,486	4,138	9,493	4,279	5,139	8,650	6,207
K	50	6,203	5,267	6,307	9,000	7,652	7,762	7,905	5,997	7,048
L	60	1,263	4,053	744	5,872	5,463	5,309	2,740	3,578	12,087
M	60	1,439	1,852	810	2,744	3,660	3,054	912	950	1,828
N	60	4,195	5,533	3,627	5,702	4,539	3,946	5,002	916	1,808
O	30	21,985	25,043	25,968	25,231	27,106	18,996	37,114	27,560	27,884
Total		124,463	113,249	138,817	147,272	154,733	153,566	133,465	155,481	188,993

"Salmon"

A	30	20,917	20,778	21,822	20,372	19,401	24,102	17,632	25,845	26,586
B	40	10,693	4,306	13,527	10,839	13,335	12,812	9,548	13,772	12,784
C	50	10,070	5,194	7,334	15,599	11,966	11,866	4,245	8,814	17,685
D	70	6,473	7,463	5,416	13,006	11,724	11,515	3,853	5,970	6,890
E	65	1,257	1,334	1,869	2,487	4,109	6,904	3,382	4,326	10,825
F	65	1,674	1,560	2,618	7,478	8,453	6,214	2,913	5,908	13,608
G	25	699	208	1,785	304	1,087	1,207	924	939	1,306
H	25	534	656	1,247	621	492	1,161	1,405	1,170	2,229
I	50	1,040	795	2,137	985	2,454	3,272	2,672	3,322	5,759
J	70	11,664	7,520	14,701	9,378	21,518	9,700	11,648	19,608	14,068
K	50	6,075	5,158	6,176	8,814	7,494	7,602	7,741	5,873	6,902
L	40	824	2,645	486	3,832	3,565	3,464	1,788	2,335	7,889
M	40	939	1,208	529	1,790	2,389	1,993	595	620	1,193
N	40	2,738	3,611	2,367	3,721	2,962	2,575	3,265	598	1,180
O	70	49,833	56,764	58,860	57,191	61,439	43,056	84,126	62,469	63,205
Total		125,430	119,200	140,874	156,417	172,388	147,443	155,737	161,569	192,109

Little Codroy salmon landed, the commercial catch data were subdivided into four groups on the basis of location: (*a*) the landings from area K (Fig. 6) near the mouth of the Little Codroy estuary; (*b*) the salmon landed at the villages of Codroy, Millville, and Searston (Fig. 1) in area K; (*c*) the landings from the remainder of area K; and (*d*) the landings from all other areas of Newfoundland and Labrador. Group *a* salmon were landed at the village of St. Andrews; the landings were small and all marked salmon were examined. Data collected by the special observers indicated that finclipped Little Codroy salmon were more concentrated in the landings at Codroy, Millville, and Searston (group *b*) than anywhere else, and that there was no excess of Little Codroy marks in the landings from group *c*. Salmon from areas F, G, H, and N (Fig. 6) in group *d* were not examined by the special observers but I have assumed that the proportion of Little Codroy salmon among those caught in these four areas was the same as in the other areas of this category.

Letting C = the estimated total numbers caught, R = the number of finclipped Little Codroy salmon reported, E = the number of fish examined by the special observers, and using the group letters defined above as subscripts (so that R_a becomes the number of finclipped Little Codroy salmon reported from category *a*, etc.), the simplest estimate of the number of Little Codroy salmon taken by the commercial fishery in Newfoundland and Labrador is:

$$R_a + [(R_b + R_c + R_d)/(E_b + E_c + E_d)]\,[C_b + C_c + C_d] \quad \ldots\ldots(1).$$

The estimate provided by equation 1 is too large because the marks are assumed to be as concentrated in categories *c* and *d* as they are in *b*. Two assumptions are necessary to allow for the special concentration of Little Codroy marks in group *b*. First, the concentration of Little Codroy marks in group *c* is assumed to be the same as in group *d*. Second, half the fish landed, and examined, in area K each year are assumed to be group *c*; this estimate is the best that can be made of this proportion. The new estimate is:

$$R_a + R_b + [(R_c + R_d)/(E_c + E_d)]\,[C_c + C_d] \quad \ldots\ldots(2).$$

The estimate provided by equation 2 is too small even if the 50:50 division of the landings from area K is correct because uninspected fish from group *b* are not included. Therefore, an unbiassed estimate would be:

$$R_a + [(R_b/E_b)\,(C_b)] + [(R_c + R_d)/(E_c + E_d)]\,[C_c + C_d] \quad \ldots\ldots(3).$$

Results

The estimated total maximum, minimum, and unbiassed number of Little Codroy salmon landed, calculated by equations 1, 2, and 3, are 1325, 867, and 1793 salmon, respectively (Tables X, XI, and XII). The unbiassed estimate is larger than the maximum estimate; theoretically it should lie between the minimum and maximum estimates. It has already been noted

TABLE X. The estimated maximum number of Little Codroy Atlantic salmon caught by the commercial fishery in Newfoundland and Labrador, 1955–63.

TABLE X. The estimated maximum number of Little Codroy Atlantic salmon caught by the commercial fishery in Newfoundland and Labrador, 1955–63.

	Sea age	1955	1956	1957	1958	1959	1960	1961	1962	1963	Total
$C_b+C_c+C_d$	.1	124,463	113,249	138,817	147,272	154,733	153,566	133,465	155,481	188,993	1,310,039
	.2+	125,430	119,200	140,874	156,417	172,388	147,443	155,737	161,569	192,109	1,371,167
$E_b+E_c+E_d$	.1	31,571	8,835	17,101	21,250	14,098	14,931	31,854	21,274	32,793	193,707
	.2+	39,548	109,073	57,031	57,785	47,703	18,135	63,082	52,906	58,986	504,249
$R_b+R_c+R_d$	.1	1	5	12	6	6	–	13	–	–	43
	.2	–	8	23	86	42	2	–	15	38	214
	.3	–	–	–	1	3	1	2	–	1	8
	PS[a]	–	–	–	1	–	–	–	1	2	4
	Total	1	13	35	94	51	3	15	16	41	269
$\frac{R_b+R_c+R_d}{E_b+E_c+E_d} \times 10^{-5}$	.1	3.167	56.593	70.171	28.235	42.559	–	40.811	–	–	–
	.2	–	7.334	40.329	148.828	88.045	11.028	–	28.352	64.422	–
	.3	–	–	–	1.730	6.289	5.514	3.170	–	1.695	–
	PS	–	–	–	1.730	–	–	–	1.890	3.391	–
$\frac{R_b+R_c+R_d}{E_b+E_c+E_d} \times (C_b+C_c+C_d)$	.1	4	64	97	42	66	–	54	–	–	327
	.2	–	9	57	233	152	16	–	46	124	637
	.3	–	–	–	3	11	8	5	–	3	30
	PS	–	–	–	3	–	–	–	3	6	12
	Total	4	73	154	281	229	24	59	49	133	1,006
R_a	.1	34	33	44	15	10	–	27	–	–	163
	.2	–	39	16	54	15	14	–	–	–	138
	.3	–	–	2	–	3	2	1	–	–	8
	PS	–	1	1	4	1	2	1	–	–	10
	Total	34	73	63	73	29	18	29	–	–	319
$R_a+\frac{R_b+R_c+R_d}{E_b+E_c+E_d} \times (C_b+C_c+C_d)$	.1	38	97	141	57	76	–	81	–	–	490
	.2	–	48	73	287	167	30	–	46	124	775
	.3	–	–	2	3	14	10	6	–	3	38
	PS	–	1	1	7	1	2	1	3	6	22
	Total	38	146	217	354	258	42	88	49	133	1,325

[a]Previously spawned.

TABLE XI. The estimated minimum number of Little Codroy Atlantic salmon caught by the commercial fishery in Newfoundland and Labrador, 1955–63

	Sea age	1955	1956	1957	1958	1959	1960	1961	1962	1963	Total
$C_b+C_c+C_d$	.1	124,463	113,249	138,817	147,272	154,733	153,566	133,465	155,481	188,993	1,310,039
C_b	.1	3,102	2,634	3,154	4,500	3,826	3,881	3,952	2,998	3,524	31,571
C_c+C_d	.1	121,361	110,615	135,663	142,772	150,907	149,685	129,513	152,483	185,469	1,278,468
$C_b+C_c+C_d$	.2+	125,430	119,200	140,874	156,417	172,388	147,443	155,737	161,569	192,109	1,371,167
C_b	.2+	3,038	2,579	3,088	4,407	3,747	3,801	3,870	2,936	3,451	30,917
C_c+C_d	.2+	122,392	116,621	137,786	152,010	168,641	143,642	151,867	158,633	188,658	1,340,250
$E_b+E_c+E_d$	.1	31,571	8,835	17,101	21,250	14,098	14,931	31,854	21,274	32,793	193,707
E_b	.1	281	424	1,122	1,016	1,782	92	338	872	798	6,725
E_c+E_d	.1	31,290	8,411	15,979	20,234	12,316	14,839	31,516	20,402	31,995	186,982
$E_b+E_c+E_d$	.2+	39,548	109,073	57,031	57,785	47,703	18,135	63,082	52,906	58,986	504,249
E_b	.2+	262	1,512	824	459	2,296	374	173	400	294	6,603
E_c+E_d	.2+	39,286	107,552	56,207	57,326	45,407	17,761	62,909	52,506	58,692	497,646
R_c+R_d	.1	1	3	3	1	1	–	2	–	–	11
	.2	–	2	8	39	12	2	–	9	22	94
	.3	–	–	–	–	1	–	1	–	–	2
	PS[a]	–	–	–	–	–	–	–	1	–	1
	Total	1	5	11	40	14	2	3	10	22	108

$\frac{E_c+R_d}{E_c+E_d} \times 10^{-5}$	.1	3.196	35.668	18.775	4.942	8.120	–	6.346	–	–	–
	.2	–	1.860	14.233	68.032	26.428	11.261	–	17.141	37.484	–
	.3	–	–	–	–	2.202	–	1.590	–	–	–
	PS	–	–	–	–	–	–	–	1.904	–	–
$\frac{R_c+R_d}{E_c+E_d} \times (C_c+C_d)$	.1	4	39	25	7	12	–	8	–	–	95
	.2	–	2	20	103	44	16	–	27	71	283
	.3	–	–	–	–	4	–	2	–	–	6
	PS	–	–	–	–	–	–	–	3	–	3
	Total	4	41	45	110	60	16	10	30	71	387
R_a+R_b	.1	34	35	53	20	15	–	38	–	–	195
	.2	–	45	31	101	45	14	–	6	16	258
	.3	–	–	2	1	5	3	2	–	1	14
	PS	–	1	1	5	1	2	1	–	2	13
	Total	34	81	87	127	66	19	41	6	19	480
$R_a+R_b+\frac{R_c+R_d}{E_c+E_d} \times (C_c^a+C_d)$	.1	38	74	78	27	27	–	46	–	–	290
	.2	–	47	51	204	89	30	–	33	87	541
	.3	–	–	2	1	9	3	4	–	1	20
	PS	–	1	1	5	1	2	1	3	2	16
	Total	38	122	132	237	126	35	51	36	90	867

[a]Previously spawned.

TABLE XII. The estimated unbiassed number of Little Codroy Atlantic salmon caught by the commercial fishery in Newfoundland and Labrador, 1955–63.

	Sea age	1955	1956	1957	1958	1959	1960	1961	1962	1963	Total
C_c+C_d	.1	121,361	110,615	135,663	142,772	150,907	149,685	129,513	152,483	185,469	1,278,468
	.2+	122,392	116,621	137,786	152,010	168,641	143,642	151,867	158,633	188,658	1,340,250
E_c+E_d	.1	31,290	8,411	15,979	20,234	12,316	14,839	31,516	20,402	31,995	186,982
	.2+	39,286	107,552	56,207	57,326	45,407	17,761	62,909	52,506	58,692	497,646
R_c+R_d	.1	1	3	3	1	1	–	2	–	–	11
	.2	–	2	8	39	12	2	–	9	22	94
	.3	–	–	–	–	1	–	1	–	–	2
	PS[a]	–	–	–	–	–	–	–	1	–	1
	Total	1	5	11	40	14	2	3	10	22	108
$\frac{R_c+R_d}{E_c+E_d} \times 10^{-5}$	.1	3.196	35.668	18.775	4.942	8.120	–	6.346	–	–	–
	.2	–	1.860	14.233	68.032	26.428	11.261	–	17.141	36.484	–
	.3	–	–	–	–	2.202	–	1.590	–	–	–
	PS	–	–	–	–	–	–	–	1.904	–	–
$\frac{R_c+R_d}{E_c+E_d} \times (C_c+C_d)$	.1	4	39	25	7	12	–	8	–	–	95
	.2	–	2	20	103	44	16	–	27	71	283
	.3	–	–	–	–	4	–	2	–	–	6
	PS	–	–	–	–	–	–	–	3	–	3
	Total	4	41	45	110	60	16	10	30	71	387
R_b	.1	–	2	9	5	5	–	11	–	–	32
	.2	–	6	15	47	30	–	–	6	16	120
	.3	–	–	–	1	2	1	1	–	1	6
	PS	–	–	–	1	–	–	–	–	2	3
	Total	–	8	24	54	37	1	12	6	19	161

C_b	.1	6,203	5,267	6,307	9,000	7,652	7,762	7,905	5,997	7,048	–
	.2+	6,075	5,158	6,176	8,814	7,494	7,602	7,741	5,873	6,902	–
E_b	.1	562	847	2,243	2,032	3,565	184	675	1,744	1,596	–
	.2+	524	3,042	1,647	918	4,591	749	346	800	588	–
$\frac{R_b}{E_b} \times 10^{-5}$	.1	–	236.128	401.248	246.063	140.252	–	1,629.630	–	–	–
	.2	–	197.239	910.747	5,119.826	653.452	–	–	750.000	2,721.088	–
	.3	–	–	–	108.932	43.563	133.511	289.017	–	170.068	–
	PS	–	–	–	108.932	–	--	–	–	340.136	–
$\frac{R_b}{E_b} \times C_b$	.1	–	12	25	22	11	–	129	–	–	199
	.2	–	10	56	451	49	–	–	44	188	798
	.3	–	–	–	10	3	10	22	–	12	57
	PS	–	–	–	10	–	–	–	–	23	33
	Total	–	22	81	493	63	10	151	44	223	1,087
R_a	.1	34	33	44	15	10	–	27	–	–	163
	.2	–	39	16	54	15	14	–	–	–	138
	.3	–	–	2	–	3	2	1	–	–	8
	PS	–	1	1	4	1	2	1	–	–	10
	Total	34	73	63	73	29	18	29	–	–	319
$R_a + \left[\frac{R_b}{E_b} \times C_b\right] +$	.1	38	84	94	44	33	–	164	–	–	457
	.2	–	51	92	608	108	30	–	71	259	1,219
$\left[\frac{R_c+R_d}{E_c+E_d} \times (C_c+C_d)\right]$	.3	–	–	2	10	10	12	25	–	12	71
	PS	–	1	1	14	1	2	1	3	23	46
	Total	38	136	189	676	152	44	190	74	294	1,793

[a]Previously spawned.

that 59 samples were discarded from the commercial catch data because of age–weight discrepancies. Even so the basic data still contain invalid observations. It is impossible to state which of the samples that have been retained are in error; it is equally impossible to know whether the missing fins were correctly identified on the samples that were discarded. Likely sources of discrepancy in the samples that were retained can be found by examining the marks to examined ratios, $(R_c + R_d)/(E_c + E_d)$, and R_b/E_b, in Table XII. Most of the ratios from the two groups seem reasonable and may be considered to form two normal populations, one for $(R_c + R_d)/(E_c + E_d)$ ratios, and the other for R_b/E_b ratios. Four ratios seem unduly large. One is in $(R_c + R_d)/(E_c + E_d)$, namely, 68.0×10^{-5} (1958 age-.2 salmon), and three are in R_b/E_b, namely, 1629.6×10^{-5} (1961 grilse), 5119.8×10^{-5} (1958 age-.2 salmon), and 2721.1×10^{-5} (1963 age-.2 salmon). Each of these differs from comparable ratios to a degree that the chance of them being a member of the same "population" is less than 1 in 10^6.

UNADJUSTED ESTIMATES OF ADULT RETURNS

The returns of finclipped Little Codroy adults, unadjusted for the effects of handling and marking, are given in Table XIII and Fig. 7 and 8. The estimated average rate of return was 2.30% and it ranged from 1.73 to 3.75% (Fig. 7). The overall average rate of return to the fence was 0.82%, and the estimated average minimum rate of return to the commercial fishery was 1.47%.

The average return of smolts as grilse was 0.96% and these were about equally divided between the fishery and the fence (Fig. 7). The average return as age-.2 salmon, 1.21%, was a little higher than that of grilse, and an estimated 0.93% were taken by the fishery and 0.28% were recorded at the fence. The weighted average return of age-.3 salmon was 0.075%, much lower than ages-.1 and .2 salmon, and the survivors were about equally divided between the fence and the fishery.

A few of the fish marked, 0.032%, were first recorded as previously spawned salmon. Four such were counted at the fence (Table III) and an estimated 16 in the commercial fishery (Table XI).

The rate of return to the fence of each sea age of adults was rather consistent (Fig. 7). On the average, 0.48% of the smolts that migrated were subsequently recorded at the fence as grilse, 0.28% as age-.2 salmon, and 0.042% as age-.3 salmon. An analysis of variance indicated that the differences between years at each sea age are not significant, and in fact quite small.

Rates of return in terms of the age at migration (smolt age), shown in Fig. 8, are based on data in Table XIII. The smolt age composition of finclipped adults at the fence and in R_a and R_b is known but the smolt age composition of the estimated returns in R_c and R_d was pro-rated from the actual returns.

The average rate of return of age-2. smolts was 1.79% as compared with 2.43% for age-3. smolts (Fig. 8). Few age-4. smolts migrated, and the weighted average rate of return, 1.64%, is based on an estimated 22 recaptures (Table

The counting fence, the commercial nets near the mouth of the Little Codroy estuary (R_{ad}), the commercial nets near Codroy, Millville, and Searston (R_b), the estimated returns from the commercial fishery in the remainder of Newfoundland and Labrador (R_c+R_d), and the estimated total returns.

the counting fence, the commercial nets near the mouth of the Little Codroy estuary (R_a), the commercial nets near Codroy, Millville, and Searston (R_b), the estimated returns from the commercial fishery in the remainder of Newfoundland and Labrador (R_c+R_d), and the estimated total returns.

Smolt run	Estimated no. of each age finclipped and released	Smolt age	Returns from commercial nets near mouth Little Codroy estuary (R_a)					Returns from Codroy, Millville, and Searston (R_b)					Estimated returns from remainder of Newfoundland and Labrador (R_c+R_d)					Estimated returns from Newfoundland and Labrador ($R_a+R_b+R_c+R_d$)					Returns from Maritimes	Returns from Gander River	Returns from Little Codroy counting fence					Estimated total returns unadjusted for effects of handling and marking				
			.1[a]	.2	.3	PS[b]	T[c]	.1	.2	.3	PS	T	.1	.2	.3	PS	T	.1	.2	.3	PS	T	.2	.1	.1	.2	.3	PS	T	.1	.2	.3	PS	T
1954	5,185	2.	14	13	2	1	30	–	5	–	–	5	–	1	–	–	1	14	19	2	1	36	–	–	15	9	2	–	26	29	28	4	1	62
	6,749	3.	19	25	–	–	44	–	1	–	–	1	–	1	–	–	1	19	27	–	–	46	–	–	71	20	–	–	91	90	47	–	–	137
	96	4.	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
		?	1	1	–	–	2	–	–	–	–	–	4	–	–	–	4	5	1	–	–	6	1	–	2	–	–	–	2	7	2	–	–	9
	12,030	Total	34	39	2	1	76	–	6	–	–	6	4	2	–	–	6	38	47	2	1	88	1	–	88	29	2	–	119	126	77	4	1	208
1955	4,573	2.	8	6	–	1	15	–	4	1	–	5	26	5	–	–	31	34	15	1	1	51	–	–	19	12	3	1	35	53	27	4	2	86
	6,447	3.	23	10	–	–	33	2	11	–	–	13	13	15	–	–	28	38	36	–	–	74	–	–	37	20	1	–	58	75	56	1	–	132
	134	4.	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
		?	2	–	–	–	2	–	–	–	–	–	–	–	–	–	–	2	–	–	–	2	1	–	3	2	2	–	7	5	3	2	–	10
	11,154	Total	33	16	–	1	50	2	15	1	–	18	39	20	–	–	59	74	51	1	1	127	1	–	59	34	6	1	100	133	86	7	2	228
1956	5,184	2.	16	18	1	1	36	2	20	1	–	23	9	21	4	–	34	27	59	6	1	93	–	–	18	16	2	–	36	45	75	8	1	129
	8,785	3.	24	29	–	4	57	7	25	1	1	34	16	71	–	–	87	47	125	1	5	178	–	–	60	29	–	1	90	107	154	1	6	268
	389	4.	–	2	–	–	2	–	–	–	–	–	–	8	–	–	8	–	10	–	–	10	–	–	1	–	–	–	1	1	10	–	–	11
	43	5.	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
		?	4	5	2	1	12	–	2	–	–	2	–	3	–	–	3	4	10	2	1	17	–	3	5	–	–	–	5	12	10	2	1	25
	14,401	Total	44	54	3	6	107	9	47	2	1	59	25	103	4	–	132	78	204	9	7	298	–	3	84	45	2	1	132	165	249	11	8	433
1957	1,175	2.	1	2	1	–	4	–	8	1	–	9	–	7	–	–	7	1	17	2	–	20	–	–	3	6	2	–	11	4	23	4	–	31
	3,245	3.	12	12	1	1	26	5	22	–	–	27	7	33	–	–	40	24	67	1	1	93	–	–	22	8	6	–	36	46	75	7	1	129
	30	4.	–	–	–	–	–	–	–	–	–	–	–	4	–	–	4	–	4	–	–	4	–	–	–	–	–	–	–	–	4	–	–	4
		?	2	1	–	–	3	–	–	–	–	–	–	–	–	–	–	2	1	–	–	3	–	–	–	–	–	–	–	2	1	–	–	3
	4,450	Total	15	15	2	1	33	5	30	1	–	36	7	44	–	–	51	27	89	3	1	120	–	–	25	14	8	–	47	52	103	11	1	167

(*Continued*)

TABLE XIII. The returns of Little Codroy Atlantic salmon, by sea age and smolt age, unadjusted for the effects of handling and marking, to the counting fence, the commercial nets near the mouth of the Little Codroy estuary (R_a), the commercial nets near Codroy, Millville, and Searston (R_b), the estimated returns from the commercial fishery in the remainder of Newfoundland and Labrador (R_c+R_d), and the estimated total returns. — (*Concluded*)

Smolt run	Estimated no. of each age finclipped and released	Smolt age	Returns from commercial nets near mouth Little Codroy estuary (R_a)					Returns from Codroy, Millville, and Searston (R_b)					Estimated returns from remainder of Newfoundland and Labrador (R_c+R_d)					Estimated returns from Newfoundland and Labrador ($R_a+R_b+R_c+R_d$)					Returns from Maritimes	Returns from Gander River	Returns from Little Codroy counting fence					Estimated total returns unadjusted for effects of handling and marking				
			.1[a]	.2	.3	PS[b]	T[c]	.1	.2	.3	PS	T	.1	.2	.3	PS	T	.1	.2	.3	PS	T	.2	.1	.1	.2	.3	PS	T	.1	.2	.3	PS	T
1958	1,364	2.	1	4	1	–	6	–	–	1	–	1	–	–	2	–	2	1	4	4	–	9	–	–	4	1	–	–	5	5	5	4	–	14
	3,130	3.	8	9	–	1	18	5	–	–	–	5	12	16	–	–	28	25	25	–	1	51	–	–	12	4	3	–	19	37	29	3	1	70
	168	4.	1	–	–	–	1	–	–	–	–	–	–	–	–	–	–	1	–	–	–	1	–	–	2	–	–	–	2	3	–	–	–	3
	9	6.	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
		?	–	1	–	–	1	–	–	–	–	–	–	–	–	–	–	–	1	–	–	1	–	–	1	–	–	–	1	1	1	–	–	2
	4,671	Total	10	14	1	1	26	5	–	1	–	6	12	16	2	–	30	27	30	4	1	62	–	–	19	5	3	–	27	46	35	7	1	89
1960	1,677	2.	3	–	–	–	3	3	–	1	1	5	–	–	–	3	3	6	–	1	4	11	–	–	2	6	2	1	11	8	6	3	5	22
	5,338	3.	22	–	–	–	22	8	6	–	1	15	4	27	–	–	31	34	33	–	1	68	–	–	10	14	3	1	28	44	47	3	2	96
	150	4.	1	–	–	–	1	–	–	–	–	–	–	–	–	–	–	1	–	–	–	1	–	–	1	1	–	–	2	2	1	–	–	3
		?	1	–	–	–	1	–	–	–	–	–	4	–	–	–	4	5	–	–	–	5	–	–	1	–	–	–	1	6	–	–	–	6
	7,165	Total	27	–	–	–	27	11	6	1	2	20	8	27	–	3	38	46	33	1	5	85	–	–	14	21	5	2	42	60	54	6	7	127
1961	4,228	2.	–	–	–	–	–	–	9	–	–	9	–	48	–	–	48	–	57	–	–	57	–	–	17	11	–	–	28	17	68	–	–	85
	2,919	3.	–	–	–	–	–	–	7	–	–	7	–	23	–	–	23	–	30	–	–	30	–	–	11	14	–	–	25	11	44	–	–	55
	376	4.	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	–	–	1	–	1	–	–	1
		?	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	2	–	–	2	–	2	–	–	2
	7,523	Total	–	–	–	–	–	–	16	–	–	16	–	71	–	–	71	–	87	–	–	87	–	–	28	28	–	–	56	28	115	–	–	143
Total	23,386	2.	43	43	5	3	94	5	46	5	1	57	35	82	6	3	126	83	171	16	7	277	–	–	78	61	11	2	152	161	232	27	9	429
	36,613	3.	108	85	1	6	200	27	72	1	2	102	52	186	–	–	238	187	343	2	8	540	–	–	223	109	13	2	347	410	452	15	10	887
	1,343	4.	2	2	–	–	4	–	–	–	–	–	–	12	–	–	12	2	14	–	–	16	–	–	4	2	–	–	6	6	16	–	–	22
	52	5.&6.	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
		?	10	8	2	1	21	–	2	–	–	2	8	3	–	–	11	18	13	2	1	34	2	3	12	4	2	–	18	33	19	4	1	57
	61,394	Total	163	138	8	10	319	32	120	6	3	161	95	283	6	3	387	290	541	20	16	867	2	3	317	176	26	4	523	610	719	46	20	1395

[a]Sea age.

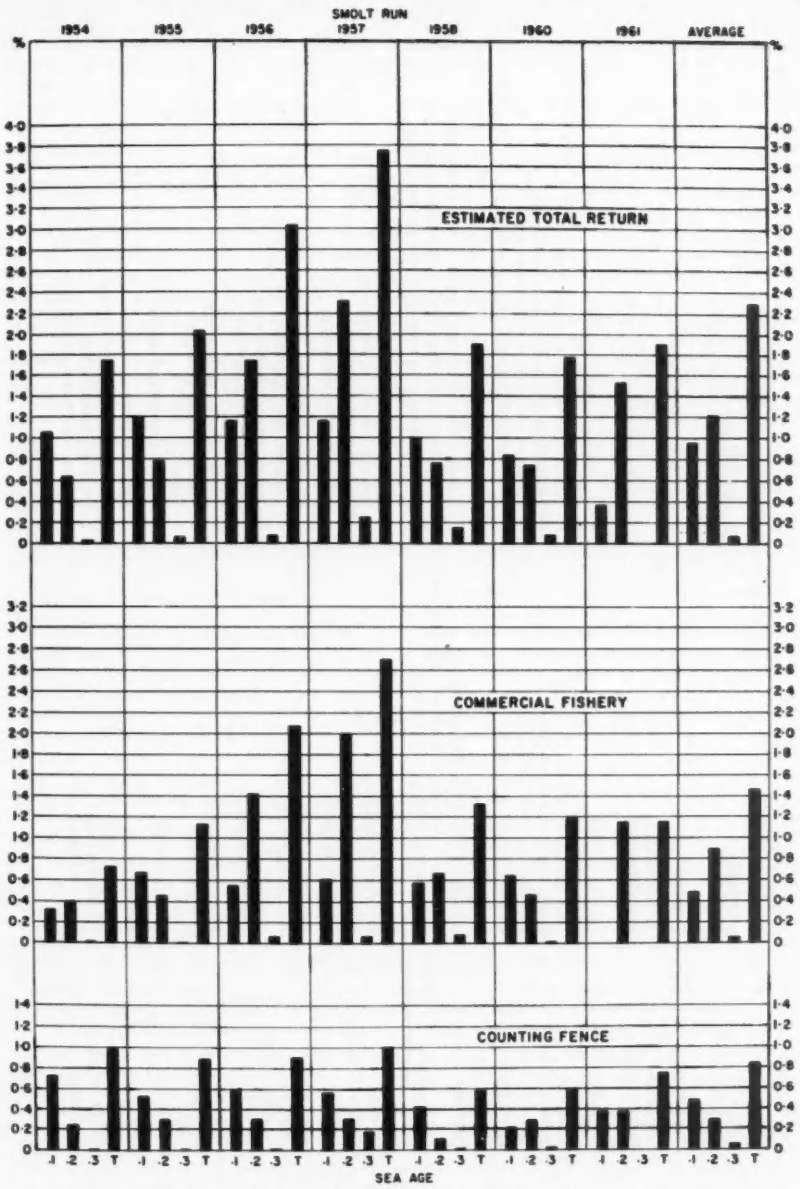

FIG. 7. Returns of finclipped Little Codroy Atlantic salmon smolts by smolt year-class and by sea age of the returning adults unadjusted for the effects of handling and marking. Rates of return of each smolt run were determined by dividing both the number of surviving adults of each sea age and the total number of surviving by the number of smolts released. The average return of ages .1 and .2 salmon and the overall total returns were calculated by averaging the percent returns giving each run equal weight (unweighted mean); the average return of age-.3 was calculated by summing the number of age-.3 survivors and dividing by the total number of smolts released (weighted mean).

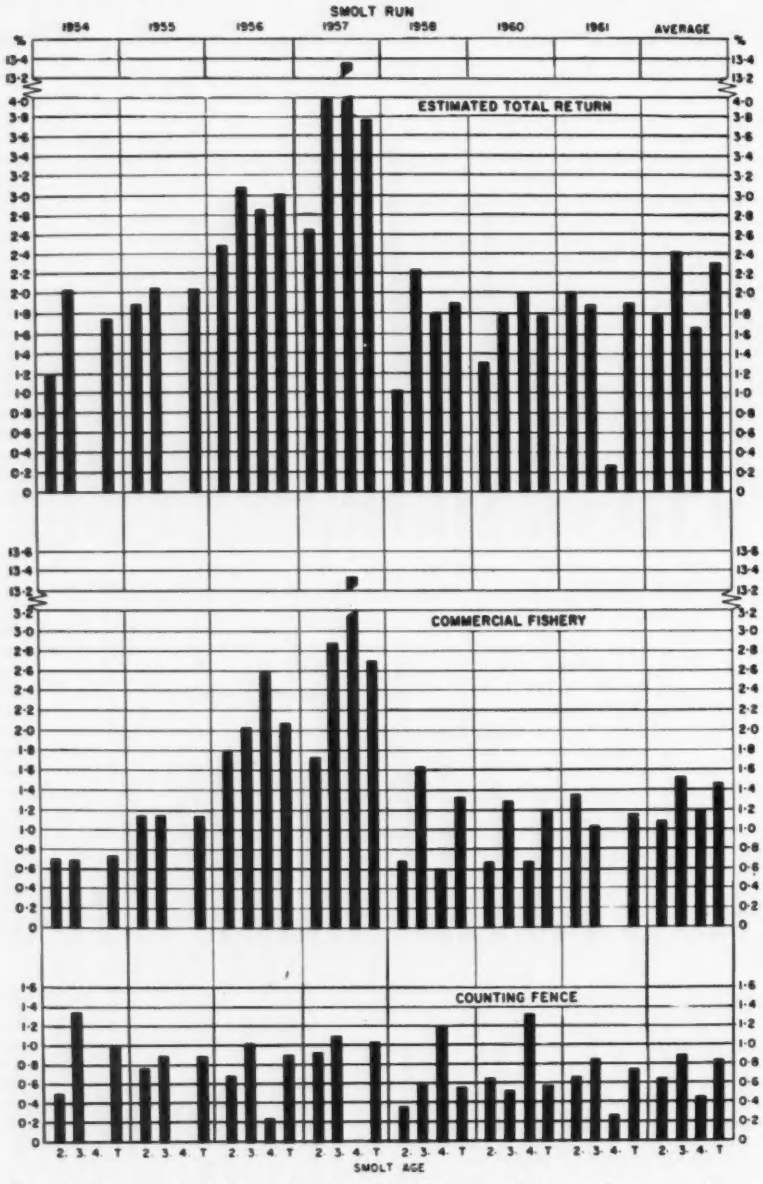

FIG. 8. Returns of finclipped Little Codroy Atlantic salmon smolts by smolt year-class and by smolt age of the returning adults unadjusted for the effects of handling and marking. Rates of return of each smolt run were determined by dividing the number of surviving adults of each smolt age by the number of smolts released which were of the same age. The average return of adults which migrated at ages 2. and 3. and the overall total return were calculated by averaging the percent returns giving each run equal weight (unweighted mean); the average return of salmon which migrated at age 4. was calculated by summing the number of survivors and dividing by the total number of age-4. smolts released (weighted mean).

XIII). At the fence, the average rate of return was 0.65% for age-2. smolts, 0.91% for age-3. smolts, and 0.44% for older smolts. In the commercial fishery, it was 1.14% for age-2. smolts, 1.52% for age-3. smolts, and 1.19% for older ones.

The smolt age composition of age-.3 adults was much different from that of adults ages .1 and .2 (Fig. 9). Only 26% of the grilse and 32% of age-.2

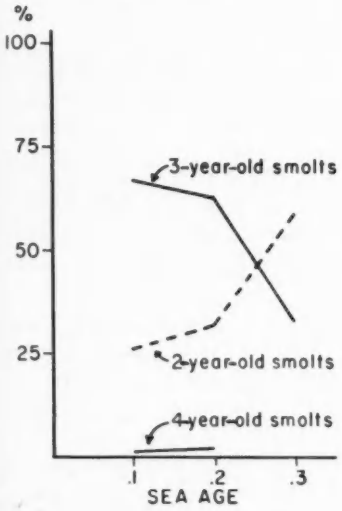

FIG. 9. Smolt age–sea age composition of surviving adult Little Codroy Atlantic salmon with each smolt age expressed as a proportion of the number recorded for each sea age.

adults, but 59% of age-.3 adults migrated at age 2.; in contrast, 67% of the grilse and 63% of age-.2 adults, but only 33% of age-.3 adults migrated at age 3. No age-4. migrants were observed among the age-.3 adults, and only about 1% of the grilse and 2% of the age-.2 adults had migrated at age 4.

ESTIMATED EFFECTS OF HANDLING AND MARKING ON SMOLT SURVIVAL

EFFECTS OF ANAESTHESIA

Smolts in the runs of 1954, 1955, 1956, and 1958 were anaesthetized with urethane to facilitate finclipping whereas smolts in the runs of 1957, 1960, and 1961 were finclipped without it (Table I). At the counting fence, grilse survival was better from runs that were anaesthetized, 0.56% as compared with 0.38%, but survival to age-.2 salmon was better from runs that were not anaesthetized, 0.33% as compared with 0.24%. A *t*-test indicates that these differences are not significant at the 5% level of probability. Therefore, in the trials of this experiment, smolt survival was not significantly affected by anaesthesia.

Effects of Marking

Estimating Procedures

The effects of marking on smolt survival were assessed by the number of adults recorded at the fence. Fence data were used because the numbers of marked and unmarked salmon were known exactly for the complete period of the experiment.

The dorsal and adipose fins were clipped from every other smolt in 1957 and 1958 to compare the survival of marked and unmarked salmon within a given run. In 1959, smolts were "handled" but not finclipped, and in 1962, smolts were neither marked nor "handled" to compare survival (Fig. 3). Identification of unmarked survivors from these four runs was difficult because some unmarked adults were observed at the fence (Table V) even when all smolts had been marked. At the time the experiments were conducted, it was believed that the unmarked adults observed at the fence were stray in the years after the smolt runs of 1954, 1955, 1956, 1960, and 1961. It was realized later, however, that the number of "stray" salmon was negligible when it was observed that the number of unmarked adults was a constant proportion of the total number of adults (Fig. 5).

Two procedures were used to assess the effects of marking. The first was based on the assumption that the number of smolts which escaped was a constant fraction of the number counted and that there was no difference in survival rates between smolts which escaped and smolts which were counted but not marked.

The following symbols are used: A = number of adults recovered at the Little Codroy fence; R = survival rate of Little Codroy smolts; S = number of Little Codroy smolts which migrated; X = average ratio of escapees to counted smolts in all years; m = finclipped salmon (used as a subscript); and u = unmarked salmon (used as a subscript).

The survival rate of finclipped smolts, R_m, is directly proportional to the ratio of the number of finclipped adults recovered, A_m, and the number of smolts marked, S_m. That is,

$$R_m = A_m/S_m \qquad \ldots\ldots(4).$$

The estimation of the survival rates of unmarked smolts, R_u, is the same but the number of unmarked adults which returned, A_u, and unmarked smolts which migrated, S_u, must be estimated from the data in the various experiments. The number of marked and unmarked adults were directly proportional in the smolt years 1954–56 and 1960–61 (Fig. 5). Hence S_m smolts were marked and S_mR_m returned. In addition, XS_m smolts escaped and XS_mR_u escapees returned. Therefore, the average ratio of escapees returned to marked returned is:

$$XR_u/R_m = XS_mR_u/S_mR_m \qquad \ldots\ldots(5).$$

Transposing:

$$XR_u = (XR_u/R_m)R_m \qquad \ldots\ldots(6).$$

In 1957 and 1958 about half the smolts were counted but not marked, S_u, and R_uS_u adults returned. In addition, $X(S_m + S_u)$ smolts escaped and $XR_u(S_m + S_u)$ adults returned. Therefore, the total number of unmarked adults which returned is:

$$A_u = R_uS_u + XR_u(S_m + S_u) \quad \ldots\ldots(7).$$

Transposing:

$$R_uS_u = A_u - XR_u(S_m + S_u).$$

Hence, the survival rate of unmarked smolts is:

$$R_u = A_u - XR_u(S_m + S_u)/S_u \quad \ldots\ldots(8).$$

By substituting equation 8 in 6, the average ratio of smolts which escaped becomes:

$$X = XR_u/R_u \quad \ldots\ldots(9).$$

The survival rates R_m and R_u are then converted to instantaneous mortality rates, and an average rate of fishing mortality subtracted from them. The loss of fins creates a handicap which may continue throughout life (Fig. 3); if so, it increases the instantaneous mortality rate by a fixed percentage each year and is best defined by the ratio of the instantaneous mortality rates of marked and unmarked fish (Ricker, 1962).

The effects of marking were also estimated from the returns of two runs in which about half the smolts were marked. Except for finclipping, marked and unmarked smolts were "handled" alike. This method too is based on the same assumptions as the one just described, namely, that the number of smolts which escaped was a constant fraction of the number counted and that there was no difference in survival rates between smolts which escaped and smolts which were counted but not marked.

The number of unmarked adults which were survivors of smolts deliberately left unmarked was calculated in the following manner. First, the observed relationship between unmarked adults at the fence and marked plus unmarked adults there (Fig. 5) was described by three linear regression coefficients, one for each sea age, in the five runs in which *all* smolts had been marked using the equation

$$b' = \Sigma(xy)/\Sigma x^2 \quad \ldots\ldots(10)$$

where b' is the linear regression coefficient, $x = A_{m+u} - \overline{A}_{m+u}$ (where A_{m+u} is the total and $\overline{A}_{m+u}$ is the average number of marked and unmarked salmon), and $y = A_u - \overline{A}_u$ (where A_u is the total and $\overline{A}_u$ is the average number of unmarked salmon). The following linear regression coefficients were calculated using data in Table V (rows 1–5): for grilse (columns 9 and 13) $b' = 0.224$; for age-.2 salmon (columns 10 and 14) $b' = 0.261$; and for age-.3 salmon (columns 11 and 15) $b' = 0.382$.

Second, the number of surviving *escapees* from the runs of 1957 and 1958 in which about *half* the smolts were finclipped was calculated using the linear regression equation

$$A_u = (\overline{A}_u - b'\overline{A}_{m+u}) + b'A_{m+u} \quad \ldots\ldots(11).$$

As an example, the following data were used to estimate the number of grilse in 1958 which had escaped marking: the average number of unmarked grilse which survived the smolt runs of 1954–56 and 1960–61 = 18.2 (Table V, row 7, column 9) = $\overline{A}_u$; the average number of marked and unmarked grilse which survived the smolt runs of 1954–56 and 1960–61 = 72.8 (Table V, row 7, column 13) = $\overline{A}_{m+u}$; the total number of marked and unmarked grilse recorded in 1958 = 80 (Table V, row 8, column 13) = A_{m+u}; and the linear regression coefficient for grilse = 0.224 = b'. Hence, by equation 11:

$$A_u = [(18.2) - (0.224)(72.8)] + [(0.224)(80)] = 20.$$

Third, the number of adults of a given sea age surviving from smolts deliberately left unmarked was determined by subtracting the number of surviving escapees (20 in the example above) from the total number of unmarked adults of the same sea age recorded at the fence (55 in the example above; see Table V, row 8, column 9). Therefore, an estimated 35 unmarked grilse, which had been handled but not finclipped as smolts in 1957, returned in 1958. A separate set of calculations was necessary for each sea age.

Rates of return for these unmarked adults were calculated by:

$$R_u = A_u/S_u \quad \ldots\ldots(12)$$

and, as before, the survival rates R_m and R_u were converted to instantaneous mortality rates. Fishing mortality rates for 1957 and 1958 smolt runs were subtracted from the instantaneous mortality rates, and, as before, the ratios of the instantaneous mortality rates of marked and unmarked fish were determined.

RESULTS

The estimated numbers which survived from smolts deliberately left unmarked are contained in Table XIV.

In Table XV an estimated immediate instantaneous mortality rate of 0.23 (see following section for method of estimation) was subtracted from the total mortality rates. Fishing mortality rates of 1.273 and 1.192 (Table XVI) were then subtracted from the 1957 and 1958 rates, respectively, to yield mortality rates other than fishing and handling. Ratios were determined, and the average, 1.106, represents the continuing handicap caused by the loss of the dorsal and adipose fins.

During the seven years 1954–58 and 1960–61, 61,394 smolts were finclipped and 523 marked adults returned to the counting fence (Table III). From equation 4, R_m, the survival rate of finclipped smolts = 523/61,394 = 0.00852.

TABLE XIV. The numbers of finclipped and nonfinclipped Atlantic salmon smolts released from the Little Codroy counting fence in 1957, 1958, 1959, and 1962, and the numbers, percentage return, and instantaneous mortality rates of finclipped and nonfinclipped[a] adults recorded at the counting fence.

	No. of smolts released			Adults recorded at the counting fence						
					Finclipped			Nonfinclipped		
		Nonfinclipped								
Smolt run	Finclipped	Handled	Not handled	Sea age	No.	Return (%)	Instantaneous mortality rate	Estimated no.[a]	Estimated return (%)	Instantaneous mortality rate
1957	4,450	4,157	–	.1	25	0.5617	5.18	35	0.8419	4.78
				.2	14	0.3146	5.76	21	0.5051	5.30
				.3	8	0.1797	6.30	0	0	–
				Total	47	1.0560	4.55	56	1.3470	4.31
1958	4,671	4,203	–	.1	19	0.4067	5.50	20	0.4758	5.34
				.2	5	0.1070	6.80	15	0.3568	5.62
				.3	3	0.0642	7.45	2	0.0475	7.65
				Total	27	0.5779	5.15	37	0.8801	4.73
1959	–	11,473	–	.1	–	–	–	33	0.2876	5.83
				.2	–	–	–	20	0.1743	6.40
				.3	–	–	–	8	0.0697	7.25
				Total	–	–	–	61	0.5316	5.24
1962	–	–	7,579	.1	–	–	–	89	1.1742	4.45

[a]These fish are survivors of smolts which were deliberately left unmarked as smolts; survivors of smolts that may have escaped are not included. See text for method of estimation.

TABLE XV. Comparison of mortality rates of marked and unmarked Little Codroy Atlantic salmon smolts, based on returns of all sea ages to the counting fence, and estimation of mortality other than fishing and handling.

Smolt run		Fence returns (%)	Instantaneous mortality rates				
			Mortality including fishing and handling	Estimated immediate (handling) mortality	Mortality other than handling	Rate of fishing (from Table XIX)	Mortality other than fishing and handling
1957	Marked	1.056	4.55	0.23	4.32	1.273	3.047
	Unmarked	1.347	4.31	0.23	4.08	1.273	2.807
	Difference	–	0.24	–	0.24	–	0.240
	Ratio	–	1.055	–	1.059	–	1.085
1958	Marked	0.578	5.15	0.23	4.92	1.192	3.728
	Unmarked	0.880	4.73	0.23	4.50	1.192	3.308
	Difference	–	0.42	–	0.42	–	0.420
	Ratio	–	1.088	–	1.093	–	1.127
Avg ratio	–	–	1.072	–	1.076	–	1.106

TABLE XVI. Percentage returns of finclipped Little Codroy Atlantic salmon, rates of exploitation, computed mortality rates, rates of survival adjusted by the average ratio of instantaneous mortality rates of marked and unmarked smolts (from Table XVIII) and by the average difference of instantaneous mortality rates of smolts handled and not handled (from Table XVII), and probable numbers of survivors.

1[a]	Smolt run:		1954	1955	1956	1957	1958	1960	1961	All years
2	Average length of smolts (*mm*):		152	153	148	140	149	149	144	150
3	Survival (%)	Fence	0.99	0.90	0.92	1.05	0.58	0.59	0.74	0.82
4		Commercial fishery	0.74	1.14	2.09	2.70	1.33	1.19	1.16	1.48
5		Total	1.73	2.04	3.01	3.75	1.91	1.78	1.90	2.30
6	Rate of exploitation		0.4277	0.5588	0.6944	0.7200	0.6963	0.6685	0.6105	0.6435
7	Instantaneous mortality rates	Gross total mortality	4.04	3.88	3.50	3.28	3.96	4.03	3.96	3.77
8		Immediate mortality	0.23	0.23	0.23	0.23	0.23	0.23	0.23	0.23
9		Net total mortality	3.81	3.65	3.27	3.05	3.73	3.80	3.73	3.54
10		Fishing mortality	0.558	0.818	1.185	1.273	1.192	1.104	0.943	1.031
11		Nonfishing mortality	3.252	2.832	2.085	1.777	2.538	2.696	2.787	2.509
12		Nonfishing mortality in absence of continuing handicap for missing fins	2.956	2.574	1.895	1.615	2.307	2.451	2.534	2.281
13		Adjusted total mortality	3.514	3.392	3.080	2.888	3.499	3.555	3.477	3.312
14	Adjusted survival (%)	Total	2.98	3.37	4.60	5.57	3.02	2.86	3.09	3.64
15		Commercial fishery	1.27	1.88	3.19	4.01	2.10	1.91	1.89	2.34
16		Fence	1.71	1.49	1.41	1.56	0.92	0.95	1.20	1.30
17	Adjusted survival (no.)	Total	358	376	662	248	141	205	232	2235
18		Commercial fishery	153	210	459	178	98	137	142	1437
19		Fence	205	166	203	69	43	68	90	798

[a]Row number.

During the five years 1954–56 and 1960–61, 52,273 smolts were marked and 448 finclipped adults returned to the fence (Table V). In addition, 52,273X smolts escaped and XS_mR_u escapees, or 133 adults, returned. From equation 5, the average ratio of escapees returned to marked returned = 133/448 = 0.297, and from equation 6, $XR_u = 0.297 \times 0.00852 = 0.00253$.

In 1957 and 1958, 9121 smolts were marked and 74 adults returned to the fence (Table V). Another 8360 smolts were counted but not marked, and $8360R_u$ unmarked adults returned. In addition, 17,481X smolts escaped and 144 unmarked adults returned (Table V). From equation 8, R_u, the survival rate of the unmarked smolts = $144 - [(0.00253 \times 17{,}481)/8360] = 0.01194$. From equation 9, X, the average ratio of smolts which escaped = 0.00253/0.01194 = 0.212.

If the counted, unmarked smolts and the escapees had different immediate mortality rates because a fixed fraction of the former was lost due to handling, then the estimated fraction of escapees, 21.2%, is too high, but the estimated return of unmarked smolts, 1.19%, remains unchanged. If, however, the counted, unmarked smolts and the escapees had different survival rates because they were of different average sizes or ages, or they returned at different ages, or they migrated at different seasons, and were thereby subjected to different mortalities, then the estimates of both S_u and X above are biassed; moreover, the bias of S_u could be in either direction.

In summary, the average fraction of escapees is estimated to be 21.2% of the total smolts in any year. Marked smolts had an average survival to the fence of 0.852% which corresponds to an instantaneous mortality rate of 4.76; unmarked smolts had an average return to the fence of 1.194% which corresponds to an instantaneous mortality rate of 4.43.

The additional mortality created by the loss of the dorsal and adipose fins, 1.097, was estimated by subtracting an average rate of fishing of 1.03 (Table XVI) from 4.76 and 4.43 and taking the ratio of the remainders. This mortality rate, 1.097, is similar to that obtained by the method used earlier, 1.106, based on the returns of smolts deliberately left unmarked.

Effects of Handling

Estimating Procedures

The effects of handling on smolt survival were estimated from two experiments. The first experiment compared the return of smolts which were counted without being taken from the water in 1962 with the returns of smolts "handled" but not marked in 1957, 1958, and 1959. It is assumed that the 1962 experiment imposed few unnatural conditions on the migrating smolts, and the survival should be comparable to that which would have resulted if no fence had been present. Differences in mortality may then be attributed to the handling procedures at the fence and it is assumed that these would affect smolts for a relatively short period of time.

Only grilse survivors from the 1962 smolt run were counted at the fence; older fish were not counted because the work ceased in 1963. It was assumed

that the same proportion of smolts escaped as formerly and that there was no difference in survival rates between smolts which escaped and smolts which were counted. The return of adults which had been counted but not marked as smolts was estimated by the method described in the previous section. Handling mortality was estimated by taking the difference between the average instantaneous mortality rates of grilse which had been handled as smolts and the instantaneous mortality rate of grilse which had not.

In the second experiment, smolts marked upstream were recaptured at the estuarial fence (Table II). The rates of recapture provided information on the effects of handling because the smolts were at liberty for a short period of time.

RESULTS

The effects of handling on survival were estimated from the 1957, 1958, 1959, and 1962 experiments. Smolts counted but not handled (1962) had an estimated rate of return of 1.17% (Table XIV). In comparison, the average survival to *grilse* of the counted, handled, and nonfinclipped survivors of the 1957, 1958, and 1959 runs was 0.54%. The additional mortality may have been caused by the handling procedures at the fence; it was calculated by taking the difference between the average instantaneous mortality rates of grilse which were handled and the instantaneous rate of grilse which were not. The difference, 0.86, is not exact and showed considerable variation from year to year in each of the three individual comparisons. It also seems rather high, and in fact has minimum reliability because it is based only on the returns of grilse; moreover, the only year of observations for the "not-handled" group was a year of exceptionally good salmon runs in Newfoundland, suggesting that sea survival may have been considerably greater than average.

Smolts marked upstream from the estuarial fence provided additional information on the effects of handling. During a 3-year period, 2395 smolts were finclipped, and 1496, or 62.5%, were captured at the fence (Table II). This is an immediate mortality of 37.5%, or 0.47 on an instantaneous basis, if none escaped through the fence. But 21.2% of each smolt run was estimated to have escaped through the fence. Assuming the same fraction of smolts escaped in these tests, then the 1496 recaptures represent a 79% recapture rate; the mortality rate now becomes 21% and the instantaneous mortality rate 0.23. This rate too is not exact; smolts were marked at two locations and the mortality rates were different: about 26% (0.30 on an instantaneous basis) of the smolts marked 13 km upstream, and 19% (0.21 on an instantaneous basis) of the smolts marked 0.4 km upstream from the fence were not recaptured. (For the figures just quoted it is again assumed that 21.2% of the smolts escaped.) The differences here may indicate that all the effects of handling and marking are not immediately apparent because fish closest to the fence had the lowest mortality.

The various tests yielded a wide range of instantaneous immediate mortality rates from handling: 0.21–0.30, average 0.23, for smolts marked within

the river; 0.24–0.42, average 0.33, for the 1957 and 1958 smolt runs (Table XV); and 0.33–1.38, average 0.86, for grilse returns from the 1957, 1958, 1959, and 1962 smolt runs. An immediate mortality rate of 0.23 was selected because it seems a reasonably accurate representation of the effects of handling smolts on the basis of marking experiments within the river. Higher values of immediate mortality may be more accurate; they would increase the adjusted returns in Table XVI but because the data contain errors and deficiencies conservative adjustments are considered more appropriate.

ADJUSTED ESTIMATES OF ADULT RETURNS

The adjusted returns of each smolt run are given in Table XVI. The gross total instantaneous mortality rates (row 7) were determined from the estimated total survival rates (row 5). An immediate instantaneous mortality rate of 0.23 was assumed to apply to all smolts and was subtracted from the gross rates. Instantaneous mortality rates due to fishing (row 10) were determined from the rates of exploitation (row 6), which, in turn, were determined by dividing the estimated survival to the commercial fishery (row 4) by the total estimated survival (row 5). Nonfishing instantaneous mortality (row 11) is the result of subtraction of items in row 10 from items in row 9. The nonfishing instantaneous mortality rates were adjusted for the continuing handicap of missing fins by dividing each by 1.10 (row 12). The adjusted total mortality (row 13) is the sum of the items in rows 10 and 12, and the corresponding adjusted survival rates are contained in row 14. The adjusted catch returns (row 15) were determined by multiplying the items in row 14 by the appropriate rates of exploitation in row 6. Fence returns (row 16) were determined by subtraction. The probable numbers of survivors (rows 17, 18, and 19) were obtained by multiplying the numbers of smolts marked (Table I, column 5) by the percentage in rows 14, 15, and 16.

The adjusted average survival is about 3.6% (Table XVI). This is 1.3% more than the 2.3% estimated to have actually survived, and is double the 1.8% recovered. In terms of numbers, it means that an estimated 2235 adult salmon would have survived from the migration of 61,394 smolts during the 7 years 1954–58 and 1960–61 if the smolts had not been handled and marked. A total of 1116 adults were actually recovered (Table III), and 1395 were estimated to have survived (Table XIII). These figures include adults at the fence, in the commercial fishery of Newfoundland and Labrador, Cape Breton, as well as the three adults angled in Gander River.

The adjusted numbers of adults for 1957 and 1958 in Table XVI are based on finclipped fish only and do not include the survivors of smolts deliberately left unmarked. The probable numbers of unmarked fish from these two runs are given in Table XVII. It is assumed that the rate of exploitation (row 5) was the same as for the finclipped fish (row 6 of Table XVI). An immediate instantaneous mortality rate of 0.23 was assumed to apply and was subtracted from the gross totals (row 6) to provide adjusted totals (row 8). The adjusted survival rates and numbers were calculated as in Table XVI.

TABLE XVII. Percentage returns of nonfinclipped Little Codroy Atlantic salmon smolts of 1957 and 1958, rates of exploitation, computed mortality rates, adjusted rates of survival, probable numbers of nonfinclipped survivors, and estimated total survivors (including the finclipped survivors in Table XVI).

1*	Smolt run:		1957	1958
2	Survival (%)	Fence	1.35	0.88
3		Commercial fishery	3.47	2.02
4		Total	4.82	2.90
5	Rate of exploitation		0.7200	0.6963
6	Instantaneous	Gross total mortality	3.03	3.54
7	mortality	Immediate mortality	0.23	0.23
8	rates	Adjusted total mortality	2.80	3.31
9	Adjusted survival (%)	Total	6.08	3.65
10		Commercial fishery	4.38	2.54
11		Fence	1.70	1.11
12	Adjusted survival (no.)	Total	253	153
13		Commercial fishery	182	107
14		Fence	71	46
15	Estimated total survival (no.)	Total	501	294
16		Commercial fishery	360	205
17		Fence	140	89

*Row number.

The estimated total survival (rows 15, 16, and 17) is the sum of the appropriate items in rows 17, 18, and 19 of Table XVI and rows 12, 13, and 14 of Table XVII. An estimated total of 501 adults would probably have survived from the 1957 smolt run, and 294 from the 1958 smolt run, if none of the smolts had been handled and marked.

ESTIMATED UTILIZATION OF LITTLE CODROY SALMON

A summary of the known utilization of finclipped Little Codroy adults is presented in Table XVIII. On the average, 62% of the known survivors, or 1.42% of the smolts marked, were estimated to have been utilized by the commercial fishery. About 4% of the known survivors, or 0.08% of the smolts marked, were taken by anglers. The remaining 34%, or 0.78% of the smolts marked, escaped and may have spawned.

The commercial fishery is estimated to have taken 47% of the grilse, 75% of the age-.2 salmon, 43% of the age-.3 salmon, and 80% of the previously spawned salmon (Table XVIII). The latter two figures are based on few returns and are not significantly different from 75%. The proportion of grilse in the landings, 33%, is lower than expected; it is believed that grilse comprise about half the landings in area K and half the total landings of

TABLE XVIII. Recorded utilization of finclipped adult Little Codroy Atlantic salmon. (See text for adjustments.)

	Sea age				
	.1	.2	.3	PS[a]	Total
Commercial fishery in Newfoundland and Labrador	290	541	20	16	867
Commercial fishery elsewhere	2	–	–	–	2
Angling on Little Codroy River	26	20	–	–	46
Angling elsewhere	3	–	–	–	3
Escapement	291	156	26	4	477
Total	612	717	46	20	1395

[a]Previously spawned.

Newfoundland and Labrador (Table IX). The fish plants prefer to handle "salmon"; many grilse are consumed locally and do not appear in the official catch statistics, and as a result, fewer grilse were available for inspection by the special observers in the plants. It is likely that *the best estimate of average rate of capture of all ages of salmon by commercial gear is the combined estimate for sea ages .2, .3, and previously spawned, namely, 73.7%*; the corresponding instantaneous rate of fishing is 1.34. If this assumption is correct, then 902 finclipped Little Codroy grilse, and not 290, were taken by the commercial fishery in Newfoundland and Labrador, and as a result, a total of 1224 grilse were utilized. The numbers of finclipped Little Codroy salmon in the Newfoundland and Labrador commercial landings are also increased, to 1479, and the proportion of grilse in them is thereby increased to 61%.

The figures just quoted are based on data unadjusted for handling and marking. The data in Tables XVI and XVII are conservatively adjusted for handling and marking but do not contain adjustments for the incomplete recording of grilse. To adjust these data, it is assumed on the basis of data in Table XVIII that 33% of the total Little Codroy commercial catch in Tables XVI and XVII were grilse. Because the total commercial catch of Little Codroy fish is 1437 salmon (Table XVI), the grilse catch is $.33 \times 1437 = 474$. But these grilse represent 47% of the grilse utilization; the remaining 53%, or 534 grilse, include fish taken by anglers and the escapement. But 73.7% of the grilse were estimated to have been utilized by the commercial fishery, therefore the 534 grilse really represent 26.3% of the utilization, and 1496 grilse, not 474, were taken by the commercial fishery. Therefore, the total commercial catch = 1496 grilse + 963 "salmon" = 2459 salmon. When this figure is added to the 798 salmon which would have been recorded at the fence (Table XVI), the total catch of Little Codroy salmon adjusted for handling and marking as well as for incomplete grilse recording, becomes 3257, or 5.30% of the 61,394 smolts that migrated.

About 4.7% of the recorded grilse and 2.6% of the older salmon were taken by anglers. If grilse are taken by commercial gear at the same rate as older salmon, their percentage take by anglers becomes 3.7% of the total grilse stock. Anglers took 9.0% of the grilse that escaped commercial capture, and 9.7% of the larger fish. Both figures are much too low to represent ordinary conditions on the Little Codroy, because of the reduced angling pressure during these experiments. Anglers took 139 salmon between 1954 and 1963, but in 1954, they took 70 salmon, 33% of the number available that year, and about half the total angling catch for the 10-year period. Angling declined markedly after 1954 because the anglers claimed that the counting fence prevented salmon from entering the river, so that between 1955 and 1963, they only took about eight salmon a year, or about 6% of what was available. If, however, they had continued to take a third of the finclipped adults available up to mid-September (the end of the angling season) each year from 1955 to 1963, they would have taken an estimated total of 146 adults, or 0.24% of the smolts that migrated. The escapement would have been reduced from 0.78 to 0.62%.

Salmon smolts were also utilized; about 12% of the smolts that were tagged in 1960 and 1961 were taken by anglers (Table II). Angling of salmon smolts is not unusual on the Little Codroy; many people, particularly children, fish for brook trout each spring when the smolts are migrating. The smolts are more abundant than the trout and readily take the lures used. The angling pressure in 1960 and 1961 did not seem to be greater than in previous years, and most of it occurred in the estuary upstream from the fence.

Only 743 of the tagged smolts, or 48% of the number tagged, were recaptured (Table II). If it is accepted that the remainder died shortly after they were released because of the capturing, handling, and tagging, then the anglers took about 25%, not 12%, of the surviving tagged smolts. There is, however, no indication that any or all of this mortality occurred before any angling pressure occurred, and 25% therefore represents a maximum estimate of the pressure exerted by anglers on the smolt run. In any case, considerable numbers of smolts are taken every spring by anglers.

RESULTS OF SOME OTHER ATLANTIC SALMON SMOLT-MARKING EXPERIMENTS

Atlantic salmon smolts have been marked in a number of rivers since 1905; the results of eight such experiments on nine Canadian and European rivers are summarized in Table XIX. A total of 110,764 smolts were marked, and at least 4940 adults, or 4.46% of the smolts, were recovered. In contrast, only 1116 adults, or 1.82% of the Little Codroy smolts, were actually recovered (Table III). Because returns to the commercial fisheries were lacking in two of the experiments in Table XIX, returns to the rivers of marking may be a more realistic way of comparing returns. A total of 2405 adults, or 2.17% of the smolts marked, returned to the nine rivers (Table XIX); only 0.82% of the Little Codroy smolts were recovered at the counting fence (Fig. 7). Returns to the Little Codroy were lower than all of the experiments reported

TABLE XIX. Numbers of Atlantic salmon smolts marked on nine Canadian and European rivers and the numbers of adult survivors reported.

Expt. no.	River	Smolts marked			Adults recaptured				Reference
					In commercial fisheries		In river of marking		
		Year	Mark	No.	*No.*	%	*No.*	%	
1	Tay, Scotland	1905	Loop of silver wire in front of dorsal fin	5,500	–	–	110	2.00	Calderwood, 1931
		1906	"fin cutting"	10,000	–	–	303	3.03	
2	Indalsälven, Sweden	1924–26	Atkins-type tag	3,751	7	0.19	6	0.16	Alm, 1931
	Ångermanälven, Sweden	1925–26	Atkins-type tag	2,575	3	0.12	6	0.23	
3	Apple, N.S.	1934	Adipose fin removed	3,252	4	0.12	113	3.47	White and Huntsman, 1938
4	Northeast Margaree, N.S.	1938	Adipose fin removed	31,359	991	3.16	1	<0.01	Huntsman, 1942
5	Moser River, Mill Brook, N.S.	1939–40	Adipose fin and X-shaped scar on left side	1,439	–	–	133	9.24	White, MS, 1940, 1941, and 1943
	Moser River, main stream	1939	Adipose fin removed	929	–	–	48	5.17	
		1940–42	Adipose fin and left ventral removed	6,108	–	–	544	8.91	
6	Lagan, Sweden	1951–54	Carlin-type tag	17,308	555	3.21	220	1.27	Carlin, 1955
7	Port Daniel, Que.	1953–55	Adipose fin removed	6,833	602	8.81	164	2.40	Bergeron, 1959
8	Miramichi, N.B.	1962	Adipose and left ventral removed	16,098	309	1.92	620	3.85	Saunders and Allen, 1967
		1962	Carlin-type tag	5,612	64	1.14	137	2.44	
Total				110,764	2,535	2.29	2,405	2.17	

in Table XIX except the Indalsälven and Ångermanälven. The difference is more marked if the data for Northeast Margaree in Table XIX are excluded because adults were not counted in that river: 79,405 smolts were marked, and 2404 adults, or 3.03% of the smolts are known to have returned. The difference is even more marked if tagged fish are excluded: 44,659 smolts were finclipped, and 1925 adults, or 4.31% of the smolts returned.

FACTORS AFFECTING SURVIVAL

Survival of Little Codroy Atlantic salmon smolts appears to have been affected not only by the capturing, handling, and marking processes, but also by such things as the mark used, and the number of smolts migrating, their size, and their age at return. Two significant results of the Little Codroy experiment are (1) an average of 0.82% of the marked smolts returned to the river (Fig. 7); and (2) the ratio of the instantaneous mortality rates of marked and unmarked fish at the fence was 1.10 (Table XV). A ratio of 1.0 means that the mark had no effect on survival (Parker et al., 1963), and a ratio of 1.10 indicates that the effect of the mark was relatively small. It is low compared with the ratios given by Parker et al. (1963) and Ricker (1962) for Pacific salmon, which ranged from 1.26 to 1.57. A return of 0.82% to the river of marking is also low for Atlantic salmon; it is 0.42–8.39% lower than the returns from five of six smolt-marking experiments summarized in Table XIX.

Marking fish causes mortality in two ways (Parker et al., 1963). Fish die a short time after release from injuries, infection, and fatigue resulting from being captured, handled, and marked. There is also long-term mortality: fish die because the mark handicaps them in escaping predators or finding food. The low survival rate and ratio of instantaneous mortality rates of marked and unmarked fish are believed to have resulted primarily from short-term mortality due to capturing, handling, and marking smolts in the Little Codroy estuary.

The Marking Site and the Resultant Capturing and Handling Methods

The choice of the estuary as the fence site was the result of the selection of the river. The Little Codroy was selected on the basis of the number of fish caught by anglers, and its size, location, and accessibility. Accessibility was particularly important. In 1953, the year in which the river was selected, the Little Codroy was perhaps the most accessible river in Newfoundland, and because it was also small, a crew of four or five men could both operate a counting fence and study the resident fish populations in upstream areas. The fence was placed in the estuary, downstream from all tributaries (Fig. 2), to get counts of all the smolts migrating from the river and all the adults returning to it. The only other place suitable for a counting fence was in Northern Brook (Fig. 2), 13 km upstream from the estuarial fence, with five of the seven major tributaries in the system downstream from the site.

Smolts captured by, and handled at, the estuarial fence were subjected to violent exercise and exposure to air, two things which Parker et al. (1963) suggest cause fatigue and death. From 1954 to 1960, smolts were caught in a trap which was set about midstream where the depth ranged from about 1.8 to about 3.3 m (Murray, 1968b). An elevator was required to lift the smolts to the top of the trap, about 1 m on the average above the river surface, so the men on the work stage could reach them. The men worked as quickly as possible to minimize exposure and care was taken to avoid crowding the smolts in the tub and subjecting them to violent exercise. But smolts were crowded in the elevator, they struggled violently, and they were exposed to air for periods up to about 30 sec while being hoisted with the elevator in the air and transferred to a tub of water. Death probably resulted from fatigue caused by struggling and exposure to air, and from osmoregulatory distress caused by scale loss due to struggling in the elevator.

In 1962, an effort was made to eliminate factors which would injure and fatigue smolts. They were slowly brought to the elevator in the trap with a seine, and they were counted without being handled or exposed to the air. However, the ratio of the instantaneous mortality rate of marked and unmarked fish is low compared with ratios for Pacific salmon quoted earlier and it is possible that the smolts could have been fatigued even by these procedures. This work was done in an estuary; in contrast, all of the smolts in the experiments in Table XIX were captured, handled, and marked in fresh water. Perhaps osmoregulation is more easily upset, and fatigue more easily caused, when smolts are in brackish water.

The Nature of the Mark

The percentage return of finclipped Little Codroy salmon to the counting fence was lower than the returns of finclipped salmon reported in Table XIX. The nature of the mark may be another important difference: 12,453 smolts were marked by the removal of their adipose fins only (the smolts finclipped on Northeast Margaree are excluded), and 458 adults, or 3.68% of the smolts returned to the rivers of marking; 22,206 smolts were marked by the removal of their adipose and left ventral fins, and 1164 adults, or 5.24% of the smolts returned to the rivers of marking. In contrast, only 0.82% of the Little Codroy smolts which were marked by the removal of their dorsal and adipose fins were recovered at the fence (Fig. 7). Excising the dorsal fin to the level of the flesh on the back of a smolt leaves a long, open wound exposing the muscles, rays, and cartilages which support the dorsal fin. Even though care was taken some smolts struggled more than usual and some of the flesh on the back beneath the dorsal fin was also cut away. The removal of the dorsal fin created, therefore, an open wound which exposed the fish to infection and no doubt contributed to death along with the osmoregulatory distress just described. In other words, immediate mortality was high, perhaps higher than the instantaneous mortality rate of 0.23 used in Tables XVI and XVII to adjust survival. If, for example, an immediate instantaneous mortality rate of 0.86

(the difference between the average instantaneous mortality rates of grilse which were handled and the instantaneous rate of grilse which were not) was used, the overall average adjusted survival rate becomes 6.46%, and if adjustments are made for grilse which were caught but not recorded, the overall average survival rate becomes 9.42%. However, as stated earlier, the use of the more conservative estimate is justified in view of errors and deficiencies in both the basic data and the estimates of the number landed.

Smolts which had been marked by the removal of their dorsal and adipose fins had an average survival rate at the fence of 0.82% (Fig. 7), 0.07% lower than that of smolts which were "handled" but not finclipped. The ratio of the instantaneous mortality rate of marked and unmarked fish averaged 1.10 (Table XV) so marked fish had a slightly higher mortality than unmarked fish. Similarly, smolts which were "handled" but not marked had a survival rate to the grilse stage of 0.54%, 0.63% lower than that of smolts which were not "handled" (Table XIV). The ratio of the instantaneous mortality rates of smolts which were "handled" to smolts not "handled" is about 1.20, though admittedly this is a very crude estimate. Nevertheless, smolts which were not "handled" may have had about the same advantage over those that were "handled" as the latter had over smolts that were finclipped.

Survival could also be affected by fin regeneration and by the natural absence of the dorsal and adipose fins. Adults with regenerated fins, if recorded as unmarked fish, would raise the survival rate and depress the ratio of instantaneous mortality rates of marked and unmarked fish. Between 1955 and 1963, 1073 Atlantic salmon adults were observed at the Little Codroy fence but only 523 were marked (Table III). Sixteen of the latter had improperly clipped fins; the dorsal fin was partially present on six, the adipose partially present on seven, and both the dorsal and adipose partially present on three. In each case, only a stub of a fin was present and the edge retained the smooth regular appearance of a fin which had been cut. An occasional smolt struggled more than usual or wriggled free as it was being clipped and so escaped without being properly clipped. The 16 improperly marked adults are presumed to be the survivors of such fish. The dorsal and adipose fins on the remaining 550 adults appeared normal and these fish were judged not to have been marked. Because the incidence of improperly clipped fish was a relatively low 3%, fin regeneration is not considered to be a factor causing low survival.

Salmon lacking one or more fins, either because they did not develop, or because they disappeared as a result of injury or infection, are not unknown (Fry, 1961). Such fish, if abundant enough, could raise the apparent survival rate of a smolt-marking experiment. However, Fry suggests that an experiment involving the removal of two fins is less likely to be affected by these so-called "natural" marks because the chance of finding one whose, say, dorsal and adipose fins are "naturally" absent is so small that it can be ignored.

The Size, Age, and Number of Smolts

Fewer marked Little Codroy Atlantic salmon smolts survived than unmarked but the ratio of the instantaneous mortality rate of marked and unmarked fish was only 1.10 (Table XV). In contrast, ratios for Pacific salmon smolts ranged from 1.26 to 1.57 (Ricker, 1962; Parker et al., 1963) indicating that the marked fish are considerably more handicapped than unmarked ones. The differences may be related to the size of the smolts; Little Codroy smolts average 150 mm (Table III) and were an average of 51–59 mm longer than Cultus sockeye, *Oncorhynchus nerka* (Walbaum), smolts of 1927–31 (Ricker, 1962). The larger fish may be less handicapped by missing fins when marked than smaller fish.

The size of the smolts at migration, the numbers migrating, and the age at return also appear to have affected survival. There were 36,613 age-3. smolts (Table III), more than any other age-group, and they had the best average survival rate, 2.43% (Fig. 8). Only 52 age-5. and 6. smolts migrated, but no adults with these smolt ages were recorded (Table III). There were 1343 age-4. smolts (Table III), and the survival rate for these fish was only 1.64% (Fig. 8). Age-2. smolts, with an average fork length of 144 mm (Table III), 9 mm less than that of age-3. smolts, had a survival rate of 1.79% (Fig. 8), 0.64% less than that of age-3. smolts. Age-4. smolts, on the other hand, with an average fork length of 160 mm (Table III), 7 mm longer than that of age-3. smolts, had a survival rate of 0.79% less than that of age-3. smolts. The age-2. smolts tended to return in greater proportion at the older sea ages, and the age-3. migrants at the younger sea ages (Fig. 9); therefore, the survival of the latter is greater than the former. The numbers migrating also seems important. It has already been noted that 52 age-5. and 6. smolts migrated and that no adults with these smolt ages were recorded. Moreover, only 1343 age-4. smolts migrated, compared with 36,613 age-3. smolts, and despite the fact that age-4. smolts were an average of 7 mm longer than age-3. smolts, their survival rate was 0.64% less than that of age-3. smolts. The survival of age-3. smolts was also affected by the number migrating: in 1961, only 2919 of them migrated whereas 4220 age-2. smolts migrated; the survival of the age-3. smolts was 1.88%, 0.13% less than that of the age-2. migrants.

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REFERENCES

ALM, GUNNAR. 1931. Resultaten av laxmärkningarna i Sverige åren. 1924–1926. Ny Svensk Fiskeritidskrift, **1**: 1–6.

BERGERON, J. 1959. Le saumon de la Rivière Port-Daniel. Actualités Marines, **3**(3): 18–24.

BLAIR, A. A. 1956. Atlantic salmon tagged in east coast Newfoundland waters at Bonavista. J. Fish. Res. Bd. Canada, **13**(2): 219–232.

1965. Bay of Islands and Humber River Atlantic salmon investigations. Ibid., **22**(2): 599–620.

MS, 1966. Atlantic salmon of St. George's Bay, Newfoundland. Fish. Res. Bd. Canada, MS Rept. Ser. (Biol.), No. 861.

CALDERWOOD, W. L. 1931. Salmon hatching and salmon migrations. Edward Arnold & Co., London. 95 p.

CARLIN, BÖRJE. 1955. Tagging of salmon smolts in the River Lagan. Rept. Inst. Freshwater Res., Drottningholm, **36**: 57–74.

FRY, DONALD H., JR. 1961. Some problems in the marking of salmonids. Pacific Marine Fish. Comm. Bull., **5**: 77–84.

HUNTSMAN, A. G. 1942. Return of a marked salmon from a distant place. Science, **95**(2467): 381–382.

KERSWILL, C. J. 1961. The management of Atlantic salmon. *In* Resources for tomorrow, Conference background papers, Vol. 2. Queen's Printer, Ottawa. p. 823–831.

MURRAY, A. R. 1958. A direct-current electrofishing apparatus using separate excitation. Canadian Fish Culturist, No. 23. p. 27–32.

MS, 1966. A summary of the commercial Atlantic salmon of Newfoundland and Labrador examined by special observers, 1953–1963. Fish. Res. Bd. Canada, MS Rept. Ser. (Biol.), No. 885.

1967. The Atlantic salmon of Newfoundland. *In* J. R. Smallwood [ed.] The book of Newfoundland, Vol. III. Newfoundland Book Publishers (1967) Ltd., St. John's, Nfld. p. 348–363.

MS, 1968a. A counting fence of netting for Atlantic salmon in the estuary of the Little Codroy River, Newfoundland. Fish. Res. Bd. Canada, Tech. Rept. No. 38.

1968b. Estuarine net counting fence for trapping Atlantic salmon. Trans. Am. Fish. Soc., **97**(3): 282–286.

PARKER, R. R., E. C. BLACK, AND P. A. LARKIN. 1963. Some aspects of fish-marking mortality. Intern. Comm. Northwest Atlantic Fish., Spec. Publ. No. 4. p. 117–122.

RICKER, W. E. 1962. Comparison of ocean growth and mortality of sockeye salmon during their last two years. J. Fish. Res. Bd. Canada, **19**(4): 531–560.

ROUNSEFELL, G. A., AND J. L. KASK. 1945. How to mark fish. Trans. Am. Fish. Soc., **73**(1943): 320–363.

SAUNDERS, R. L., AND K. RADWAY ALLEN. 1967. Effects of tagging and of fin-clipping on the survival and growth of Atlantic salmon between smolt and adult stages. J. Fish. Res. Bd. Canada, **24**(12): 2595–2611.

STATE OF MAINE ATLANTIC SEA RUN SALMON COMMISSION. 1952. Biennial report. Augusta, Maine. 16 p.

1960. Biennial report. Orono, Maine. 14 p.

WHITE, H. C. MS, 1940. The Moser salmon run. Atlantic salmon and trout investigation, App. 17: 30–31. Ann. Rept. Atlantic Biol. Sta., Fish. Res. Bd. Canada, St Andrews, N.B. Mimeo.

MS, 1941. The salmon run. Atlantic salmon and trout investigations, App. 25: 31–32. Ann. Rept. Atlantic Biol. Sta., Fish. Res. Bd. Canada, St. Andrews, N.B. Mimeo.

MS, 1943. The return from marked smolts at Moser River. Atlantic salmon and trout investigations, App. 23: p. 35. Ann. Rept. Atlantic Biol. Sta., Fish. Res. Bd. Canada, St. Andrews, N.B. Mimeo.

WHITE, H. C., AND A. G. HUNTSMAN. 1938. Is local behaviour in salmon heritable? J. Fish. Res. Bd. Canada, 4(1): 1–18.

NOTES

Loss of Radioactivity During Storage of ^{14}C-Labelled Phytoplankton on Membrane Filters

A number of the procedures involved in the measurement of phytoplankton production with $^{14}CO_2$ are reasonably standard and in general use. One of these is the practice of storing labelled phytoplankton on membrane filters for later counting. This often eliminates the need for counting facilities on board ship or at remote field stations. Doty and Oguri (1958) reported little loss of ^{14}C activity from phytoplankton with time. However, recent work in our laboratory indicates that a substantial loss of activity may occur within the first 24 hr of storage. We have measured the storage loss of ^{14}C activity from natural populations of marine and freshwater phytoplankton as well as algal cultures.

Methods.— Surface samples of freshwater phytoplankton were obtained in the summer and fall, 1967, from Marion, Trout, and Deer lakes near Vancouver, B.C. Marine phytoplankton samples were collected from Indian Arm, also near Vancouver. All samples were collected in 5-liter polyethylene bottles, strained through nylon netting to remove the zooplankton and larger detrital particles, and stored in the laboratory in subdued light for a few hours before use.

The Marion Lake population in September 1967 consisted mainly of the myxophycean *Merismopedia limneticus* (about 60% by number), the chrysophycean *Dinobryon sertularia* (30%), *Scenedesmus* sp. (5%), and a few diatoms and dinoflagellates (5%). The Trout Lake population was primarily small diatoms, probably *Navicula* sp. The populations in Deer Lake were primarily green and blue-green algae. Euglenoids, diatoms, and dinoflagellates were present in roughly equal numbers in the Indian Arm samples.

We have also measured the loss of activity associated with storage of filtered samples of synchronously grown cultures of *Chlorella pyrenoidosa* and *Scenedesmus obliquus*. Incubation with $Na_2\ ^{14}CO_3$ was commenced 2 hr after cell division.

All samples were incubated in 300-ml BOD bottles or, occasionally, in Erlenmeyer flasks. Incubation temperature for the natural populations was that from which they were taken and for the cultures of *Chlorella* and *Scenedesmus* was 20 C. Samples were placed on a shaker (120 cycles/min) and were incubated with 10 μc of $Na_2\ ^{14}CO_3$ under a bank of eight cool white fluorescent lights (35×10^4 ergs cm^{-2} sec^{-1} at the top of the bottles). After incubation we removed from 12 to 48 10-ml aliquots from the well-mixed sample and filtered each aliquot through a Millipore HA filter (0.45-μ pore

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diam) under a vacuum of 38 cm Hg. We followed the procedure recommended by McAllister (1961) and rinsed samples on the filter with 20 ml membrane-filtered water obtained from the sampling area or culture flasks. Variation in the volume of rinse water from 5 to 20 ml did not have a large or consistent effect on the activity remaining in the plant cells. We thus obtained a series of filters with comparable activity from each sample.

The activity of the plankton on some filters was determined immediately after filtration. The remaining filters were stored for varying periods and under a variety of conditions before the activity was measured. In all cases the filters were stored in a desiccator over silica gel and a CO_2 absorbent. The desiccators, which were occasionally evacuated, were kept at either room temperature or −12 C. Activity of several stored samples from the same BOD bottle was usually measured after 24 and 48 hr. Other samples were counted after 8 weeks storage.

The activity of phytoplankton samples was determined by placing the membrane filter and labelled phytoplankton in a vial containing 15 ml of a scintillator. Each sample was counted three times for 5 min with a Packard Tri-Carb scintillation spectrometer. Background was substracted from the mean of the three counts. Two scintillators which gave comparable counts were used. Dried filters were placed in a scintillator of the following composition: 4 g of 2,5-diphenyloxazole (PPO) and 50 mg of 1,4-bis-2-(methyloxazolyl)-benzene (POPOP) were dissolved and brought to a volume of 1 liter in toluene. The dry filters became transparent in the scintillator and settled to the bottom of the vials.

A second scintillator was required for measurement of the activity of phytoplankton on damp filters. This one, referred to as TEG, was made up of 10 volumes of the previous scintillator and 6 volumes of ethylene glycol monomethyl ether. A volume of 15 ml of this scintillator will hold up to 0.3 ml water and dissolves the wet filter. Samples on dried membrane filters gave erratic counts in TEG. We were unable to find a scintillator that would give reproducible results with samples on both wet and dry filters.

We examined the possible significance of self-absorption on our counts in the following way. Several aliquots, each of a larger volume than the previous one, were drawn from the same labelled sample of phytoplankton. Each aliquot was filtered through a separate membrane filter. In this way we prepared a series of filters with a range in quantity of particulate matter and total activity. There was no indication of self-absorption in any samples requiring less than 12 min for filtration. Most samples were filtered in 2–5 min.

Counting efficiency was calculated by the channels ratio method of Peng (1966) and counts converted to absolute activity. There was no significant difference in the efficiency of a spike with or without a filter in the vial or with regard to the orientation of the filter in the counting vial.

Results and discussion.— The results demonstrate that a variable and often significant proportion of ^{14}C activity contained in phytoplankton cells

is lost when the cells are stored on a membrane filter in a desiccator. Most of this loss occurs within the first 24 hr of storage (Table I). Little change in activity was recorded between 24 and 48 hr or 8 weeks storage (Table I, III).

The loss of activity was more or less independent of storage conditions (Table I, II). However, the loss of activity, which ranged from 2.9 to 50.7% of original activity, was related to the species composition of the phytoplankton. *Chlorella* lost the smallest percentage of the original activity, *Scenedesmus* the largest.

Our results indicate that the activity of ^{14}C-labelled phytoplankton can be expected to decrease if samples are stored prior to counting. If sample

TABLE I. A comparison of the ^{14}C activity (dpm) in *Scenedesmus obliquus* and phytoplankton from Deer Lake immediately after incubation with $Na_2{}^{14}CO_3$ and after storage in desiccators on membrane filters over silica gel and a CO_2 absorbent for 24 and 48 hr. Cells assayed for activity immediately after filtration were placed in vials of TEG. Those assayed after storage were placed in vials of a toluene scintillator. Conditions of storage are shown in the body of the table.

		24-hr storage			48-hr storage		
Scenedesmus obliquus	Initial *dpm*	Room temp no vacuum *dpm*	−12 C no vacuum *dpm*	−12 C vacuum *dpm*	Room temp no vacuum *dpm*	−12 C *dpm*	−12 C evacuated *dpm*
	230,326	133,557	129,450	132,234	133,812	131,066	134,689
	229,320	135,406	134,205	133,322	133,016	134,630	131,999
	230,190	134,205	130,845	135,605	129,329	132,913	133,689
	228,639	132,321	133,691	135,747	136,210	134,628	133,601
	230,199	133,179	132,612	134,490	131,699	132,633	132,939
	220,139	135,404	135,255	134,123	132,639	134,201	128,959
Mean	228,135	134,012	132,626	134,253	132,784	133,345	132,646
SD	3,971	1,239	2,181	1,347	2,279	1,408	2,013
% loss		41.3	41.9	41.2	41.8	41.6	41.9
Deer Lake phytoplankton	Initial *dpm*	Room temp no vacuum *dpm*	Room temp vacuum *dpm*	−12 C no vacuum *dpm*	Room temp no vacuum *dpm*	Room temp vacuum *dpm*	−12 C no vacuum *dpm*
	1,039	810	839	835	792	860	685
	1,060	796	849	833	835	849	823
	1,074	796	859	794	793	889	794
	1,063	738	862	886	756	826	795
	1,170	751	828	895	760	798	867
	1,173	738	855	871	728	753	872
Mean	1,096	772	848	852	777	829	806
SD	59	33	34	32	37	48	68
% loss		25.1	22.7	21.4	24.6	21.8	21.7

TABLE II. Percentage loss of ^{14}C activity by plankton algae stored in desiccators on membrane filters for 24 hr. Each value represents the mean of six to eight observations.

	Room temp (%)	Room temp with vacuum (%)	−12 C (%)
Chlorella July 10	3.9	4.2	3.7
Chlorella July 21	4.2	5.5	2.9
Marion Lake Sept. 21	5.8	4.7	8.3
Marion Lake Sept. 31	3.6	4.3	3.0
Deer Lake July 26	25.1	22.7	21.4
Deer Lake Aug. 16	24.3	24.5	27.9
Scenedesmus Aug. 7	47.6	50.7	50.7
Scenedesmus Sept. 25	33.7	35.9	33.7
Scenedesmus Jan. 26	41.3	41.2[a]	41.9
Trout Lake Aug. 8	46.0	47.9	45.1
Indian Arm July 26	30.4	31.9	40.8
Indian Arm Sept. 27	30.7	27.1	27.9

[a]This particular sample was stored under a vacuum at −12 C.

TABLE III. Percentage loss of ^{14}C activity during storage of Indian Arm phytoplankton samples for periods up to 8 weeks. Samples were stored on dried membrane filters in a desiccator at 20 C.

	Initial *dpm*	24-hr *dpm*	48-hr *dpm*	8-weeks *dpm*
	1086	791	734	774
	1062	551	709	728
	1062	806	782	718
	1054	774	798	696
	1157	761	807	768
	1128	783	800	763
Mean	1092	744	771	741
SD	42	96	21	32
% loss		31.9	29.4	32.1

activity is measured with a Geiger counter, this should be undertaken immediately the damp sample and membrane filter are removed from the filtration unit. However, this might present additional problems in applying a self-absorption correction. We have not followed the time course of the decrease in activity within the first 24 hr, but it is possible that much of the loss occurs during the early hours of storage when water is rapidly removed from the cells by the desiccant.

Two alternative methods of handling the freshly filtered phytoplankton should eliminate the loss of activity experienced during storage. The labelled material could be combusted and the activity measured in gas phase or the labelled cells could be placed in a vial of scintillator and activity determined in a scintillation counter. We have used the latter method. It has the advantage that the damp membrane filter and phytoplankton can be placed in the scintillator immediately after filtration and stored in this way for extended periods with no measurable loss of activity. This procedure lends itself to field studies where the necessary counting equipment may not be available. It also eliminates the need to correct for self-absorption as long as the quantity of particulate matter in the scintillator is moderate. This eliminates the necessity of weighing dried membrane filters before and after filtration.

All samples should be well agitated before their activity is determined with the scintillation counter. If a large number of samples are to be counted in succession it is necessary to ensure that the cells remain in suspension, either by frequent shaking or by the addition of a gelling agent such as Cab-O-Sil. Efficiency of counting is lowered when the cells settle out.

The activity lost by the plant cells can be quantitatively recovered by placing an open container of Hyamine in the desiccator. This provides an indication of the nature of the lost activity since Hyamine, a strong base, absorbs CO_2 and acidic compounds (Passman et al., 1956). Perhaps glycollic acid, other organic acids, and some small ketones and aldehydes might decompose under the storage conditions we have employed. However, the amount of activity lost through decomposition of organic compounds is not likely to exceed 10–15% of the total activity. It is possible that the activity is lost from the plant cells as $^{14}CO_2$ derived from a pool of CO_2 or bicarbonate present in the cells. The presence of such a pool in *Chlorella* has been suggested by Bassham and Kirk (1964). The idea of such a pool in the leaves of higher plants has the suport of numerous physiologists although little has been published on this topic. Species variation in pool size, if in fact pools do exist, might be expected. This could account for the differences in the amount of activity lost on storage by the various species and populations that we have studied.

Activity lost as a result of the decomposition of labelled organic compounds will contribute to an underestimate of production rate. An error of this type might account for some of the discrepancy between the ^{14}C and oxygen light–dark bottle estimates of production.

The effect on measured rates of production of a loss of labelled intracellular carbon before reduction will be determined by the subsequent fate of any label in a pool. Let us visualize phytoplankton cells containing a pool of intracellular CO_2. If these cells are then placed in a medium containing $^{14}CO_2$ and permitted to photosynthesize, the first photosynthetic products might be produced by the reduction of unlabelled pool CO_2. The production of labelled organic compounds could be expected after the $^{14}CO_2$ was incorporated into the pool. If the intracellular $^{14}CO_2$ remaining at the end of the

experiment would have been routinely reduced to an organic form, then a reasonable production estimate is perhaps derived from the total activity incorporated by the plant cells (i.e. intracellular $^{14}CO_2$ and labelled organic compounds). The $^{14}CO_2$ activity remaining in the cell at the end of the experiment might compensate for the unlabelled CO_2 incorporated from the pool when the plants were first placed in the labelled medium. If some of this labelled $^{14}CO_2$ is lost during storage then production will be underestimated. If, on the other hand, some of the pool $^{14}CO_2$ is not reduced to an organic form in the normal course of events, we will have overestimated production of particulate matter. This line of reasoning assumes the presence of an intracellular pool of CO_2 or bicarbonate and species differences in pool size.

The methods we have used to collect, incubate, filter, and store the phytoplankton samples are similar to those described by Strickland and Parsons (1965) and are employed by numerous workers. Any errors inherent in our methods probably apply generally to the use of ^{14}C for measuring phytoplankton production. Our results suggest a possible source of error associated with the ^{14}C method that has not previously been recognized.

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REFERENCES

BASSHAM, J. G., AND M. KIRK. 1964. Kinetic studies of the photosynthetic carbon reduction cycle. Ann. Rev. Plant Physiol., **15**: 101–119.

DOTY, M. S., AND M. OGURI. 1958. Selected features of the isotopic carbon primary productivity technique. Rappt. Procès-Verbaux Réunions, Conseil Perm. Intern. Exploration Mer, **144**: 47–55.

MCALLISTER, C. D. 1961. Decontamination of filters in the C^{14} method of measuring marine photosynthesis. Limnol. Oceanog., **6**: 447–450.

PASSMAN, J. M., N. S. RADIN, AND J. A. D. COOPER. 1956. Liquid scintillation technique for measuring carbon-14-dioxide activity. Anal. Chem., **28**: 484–486.

PENG, C. T. 1966. Quenching correction in liquid scintillation counting. *In* S. Rothchild [ed.] Advances in tracer methodology. Vol. 3. Plenum Press, New York, N.Y. p. 81–94.

STRICKLAND, J. D. H., AND T. R. PARSONS. 1965. A manual of sea water analysis. Bull. Fish. Res. Bd. Canada, No. 125. 2nd ed. 203 p.

Reproduction of White Perch, *Roccus americanus*, in the Bay of Quinte, Lake Ontario

This note reports on the age and size at maturity, sex ratios, spawning season, and fecundity of white perch, *Roccus americanus*, inhabiting the Bay of Quinte area of Lake Ontario. Scott and Christie (1963) believed the white perch invaded Lake Ontario about 1950 via the Oswego River system in upper New York State. Since then, it has become very abundant in the Bay of Quinte area and in the last few years it has usurped the dominant position in the fish fauna of the Bay formerly held by the yellow perch, *Perca flavescens*. It is now fished commercially and may be of some sporting interest. During the last 10 years, the staff of the Ontario Department of Lands and Forests Fisheries Station at Glenora have been collecting information on the biology of the white perch in the Bay of Quinte. Their data and our own observations show the species matures at an early age, breeds annually for a number of years, and has a very high fecundity. Its reproductive capacity must be considered in attempting to explain the almost explosive increase in its numbers during the last decade.

Materials and methods.— Details of 3502 white perch sampled during test fishing between 1957 and 1966 were available to provide information on sex ratios and age and size at maturity. Collections were made twice monthly wherever possible.

In addition, during the spring of 1967, 50 gravid females were collected near the dock of the Fisheries Station at Glenora using Windermere traps. Each trap was 4 ft long and 2 ft in diam, with an opening on one side tapering to 4 inches, and a door on the other side from which fish could be removed. The traps were made of ½-inch wire gauze or ½-inch nylon mesh. Only ripe females which were not suspected of partial spawning were selected. These were easy to recognize as with very little pressure on the abdomen, amber-coloured eggs were extruded which stuck together and had a very thick jelly-like consistency. The females selected ranged from 123 to 281 mm fork length, weighed 36.3–485.3 g, and were I+ to VIII+ years of age as determined from the scales. The fork length, weight, and place and date of collection were recorded and scales were taken from the left side of the body, from an area above the lateral line and between the soft and spinous dorsal fin. Then each fish was opened and the complete ovaries carefully removed. The ovaries were preserved in Davidson's Solution A for 24 hr and then transfered to Davidson's Solution B until the eggs were counted (after Henderson, 1963).

Examination revealed the ovaries contained eggs of three obviously different sizes, distributed uniformly throughout the organ. As the fish were about to spawn when collected, only the large eggs would have been shed and only these were included in the counts.

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The method of counting was as follows. The ovaries from each fish were dried and weighed. Samples were removed from the anterior, middle, and posterior parts of the ovaries with the ovary wall attached. The samples, amounting to about one-tenth of the ovaries, were weighed and the eggs counted under magnification. The number of eggs per gram weight of ovary was calculated for each sample. There was no apparent difference between the three parts of the ovary. Taub (MS, 1966) stated that an analysis of variance revealed that the number of eggs per 10 mg ovary varied among fish ($P = 0.05$); it did not vary among sections within an ovary.

The fecundity of each fish was calculated from the mean number of eggs per gram of ovary in the samples and the total weight of the ovaries.

Results.— Our observations and the records of the Fisheries Station at Glenora show that the gonads of white perch inhabiting the Bay of Quinte mature in May and spawning takes place from the middle of May to the end of June. At the end of May and during the first half of June the females are partly spent. By the end of June they are completely spent. Water temperatures during the spawning period are in the range of 11–15 C.

Some white perch mature in their 2nd year of life and all are mature by the 4th year (Table I). In the sample studied 8.1% of the males were mature

TABLE I. Percentages of white perch sexually mature and sex ratios at various ages, Bay of Quinte, Lake Ontario, 1957–66.

Age	Males	% of total	% mature	Females	% of total	% mature	Male: female
I+	111	8.4	8.1	122	5.6	5.7	1:1.1
II+	223	17.0	100.0	387	17.7	76.0	1:1.7
III+	286	21.8	100.0	426	19.5	100.0	1:1.5
IV+	293	22.3	100.0	393	18.0	100.0	1:1.3
V+	213	16.2	100.0	393	18.0	100.0	1:1.8
VI+	132	10.0	100.0	257	11.8	100.0	1:2.0
VII+	45	3.4	100.0	153	7.0	100.0	1:3.4
VIII+	12	0.9	100.0	48	2.2	100.0	1:4.0
IX+	–	–	–	6	0.3	100.0	
X+	–	–	–	2	0.1	100.0	
Total	1315			2187			1:1.7

at age I+ and all males were mature at age II+; 5.7% of the females were mature at age I+, 76% at age II+, and all at age III+. All males exceeding 140 mm fork length and all females over 172 mm fork length were sexually mature. Females outnumbered males in all age-groups by a ratio of 1:1.7. The ratio of females to males tended to increase with age which suggests they experience a lower mortality rate.

In a sample of 50 fish the number of mature ova ranged from 5210 in an age-I+ fish of 123 mm fork length (the smallest fish in the collection) to 247,681 in an age-VII+ fish of 250 mm fork length (the third largest in the collection) (Table II). The mean number of ova increased as the fork length

TABLE II. Numbers of eggs per fish found in various length-groups in a sample of 50 white perch, Bay of Quinte, and ratios of mean numbers of eggs in the groups to the number (5210) in the smallest fish in the sample.

Fork length (*mm*)		No. of fish	No. of eggs per fish		
Interval	Mean		Range	Mean	Ratio
123	123	1	5,210	5,210	1:1.0
151–160	158.3	4	15,740–26,770	21,180	1:4.1
161–170	166.5	4	22,609–28,580	29,439	1:5.7
171–180	175.9	14	18,598–61,488	36,687	1:7.0
181–190	185.8	11	25,322–113,892	59,866	1:11.5
191–200	194.5	4	43,021–82,601	61,959	1:11.9
201–210	206.7	3	91,448–98,556	95,752	1:18.4
211–220	216.7	3	75,210–149,122	101,924	1:19.6
221–230	227.5	2	76,094–195,673	135,883	1:26.1
231–240	–	–	–	–	–
241–250	249.0	2	221,003–247,681	234,342	1:45.0
265	265	1	146,503	146,503	1:28.1
231	281	1	117,624	117,624	1:22.6

of the fish increased but considerable variability existed amongst fish of similar size. The ratio of the mean egg production of fish in the various length-groups compared with that of the smallest fish increased rapidly. The 241–250-mm group was double the length of the smallest fish but produced more than 44 times as many eggs. In the largest two fishes, there was a decrease in the ratio. Such a decrease, if based on an adequate sample, would indicate a decrease in the rate of egg production in the largest and oldest fish. The relationship between fecundity and fork length was expressed as the linear regression $Y = 2.912 + 0.009\,X$ ($r = 0.82$) where Y is the logarithm of the number of eggs and X is the fork length in millimeters (Fig. 1).

The sample contained only one fish in the 2nd year of life (age-group I+) and one in the 3rd year. The mean number of eggs increased from 5210 in age-group I+ to 170,602 in age-group VIII+. The number of eggs varied considerably between fish in the same age-group. The relationship between fecundity and age was expressed as the linear regression $Y = 4.076 + 0.126\,X$ ($r = 0.51$) where Y = the logarithm of the number of mature eggs and X is the age-group with the plus sign omitted (Fig. 1).

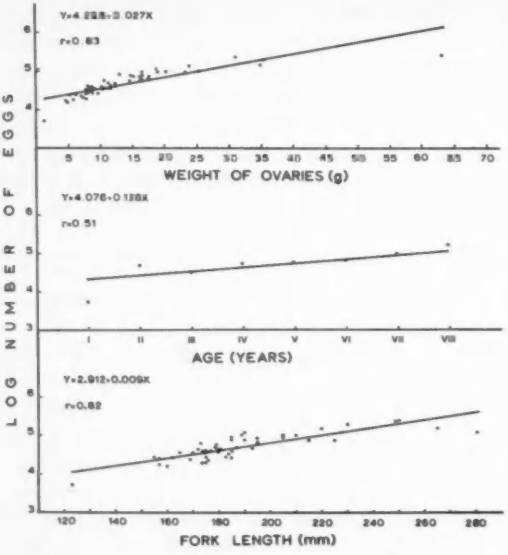

FIG. 1. Relationship between log number of eggs and fork length, age, and weight of ovaries in a sample of 50 female white perch from Bay of Quinte, Lake Ontario.

Fecundity was not so clearly related to weight (r = 0.00005) as to length or age. Fecundity ranged from 5210 at weight 36.3 g to 221,003 at weight 308.4 g (Table IV). The mean number of eggs produced by fish grouped in 20-g weight intervals showed an irregular increase and considerable overlapping in the ranges. There was also much variation in the number of eggs produced per gram body weight.

A relationship established between fecundity and weight of the ovaries was described by the equation Y = 4.298 + 0.027 X (r = 0.83) where Y =

TABLE III. Fork lengths, numbers of eggs per fish, and weights of fish according to age in a sample of 50 white perch, Bay of Quinte.

Age	No. of fish	Fork length (*mm*)		No. of eggs per fish		Weight of fish (g)	
		Range	Mean	Range	Mean	Range	Mean
I+	1	–	123.0	–	5,210	–	36.3
II+	1	–	187.0	–	47,384	–	131.5
III+	2	157–165	161.0	24,965–38,580	31,772	72.57– 95.25	83.9
IV+	12	170–225	187.8	18,598–149,122	54,392	86.18–249.47	141.6
V+	22	155–243	185.9	15,740–221,003	58,621	77.11–308.44	127.8
VI+	6	169–210	185.3	22,609–113,892	65,086	86.18–176.90	130.8
VII+	3	174–230	196.3	37,098–195,673	102,387	99.78–290.29	175.4
VIII+	3	250–281	265.3	117,624–247,681	170,602	254.01–485.34	361.3

TABLE IV. Numbers of eggs per fish according to weight and numbers of eggs per gram of fish in a sample of 50 white perch, Bay of Quinte.

Weight of fish (g)		No. of fish	No. of eggs per fish		No. of eggs per gram of fish	
Interval	Mean		Range	Mean	Range	Mean
36.28	36.2	1	–	5,210	–	143
61–80	74.3	5	15,740–26,770	21,995	223–347	279
81–100	94.7	9	18,598–39,960	28,640	186–463	303
101–120	108.5	7	25,322–61,488	38,827	223–542	354
121–140	129.6	12	36,528–97,877	54,663	287–799	420
141–160	148.5	4	60,178–113,892	82,530	414–717	551
161–180	172.3	3	75,210–98,556	90,339	425–603	525
181–200	–	–	–	–	–	–
201–220	206.3	2	81,441–91,448	86,444	399–438	418
231.33	231.3	1	–	76,094	–	328
241–260	251.7	2	149,122–247,681	198,401	597–975	786
290.29	290.2	1	–	195,673	–	674
308.44	308.4	1	–	221,003	–	716
344.72	344.7	1	–	146,503	–	424
485.34	485.3	1	–	117,624	–	242

the logarithm of the number of mature eggs and X is the weight of the ovaries in grams (Fig. 1). The weight of the 50 ovaries studied varied from 1.5 to 63.2 g and the number of ova per gram of ovary ranged from 2687 to 7084.

Discussion.— Our results show that the reproductive performance of white perch in the Bay of Quinte is similar to that elsewhere in the range. Spawning occurs at water temperatures between 11 and 15 C. Because of latitude it is later than reported in the literature, which deals with populations living further south in the United States, many of which are anadromous (Hildebrand and Schroeder, 1928; Hoover, 1937; Bigelow and Schroeder, 1953; AuClair, MS, 1956; Conover, MS, 1958; Thoits, 1958; Mansueti, 1961; Miller, MS, 1963; Taub, 1966). Bay of Quinte fish mature at ages I+ to III+ years, the same ages as reported by Truitt (1952), Thoits (1958), Miller (MS, 1963), and Mansueti (1961, 1964).

There is considerable variation in the published estimates of egg production. AuClair (MS, 1956), using a volumetric method, estimated the number of eggs in an 18-oz fish as 160,000 which he believed was conservative. Using a volumetric method, he found the average number of eggs per pound of fish to be 145,920. This was an average obtained from 19 gravid females ranging in weight from 4 to 23 oz, mean weight 13 oz. Conover (MS, 1958) found 90,167 eggs in an age-III fish 224 mm long. This was the maximum of 13 ovaries he counted. Taub (MS, 1966) gave the mean fecundity of age-groups III–VI as 271,000 ova, the range being 190,000–321,000. Some of the variability is undoubtedly related to the size of females selected for counts. Our own

results clearly show how greatly the fecundity changes with increase in length and age. In addition, we found considerable variation in fecundity between fish of similar size and age, and therefore a large sample must be studied for an accurate assessment of the reproductive potential of a population.

Interest in the white perch in the Bay of Quinte was prompted by the explosive increase in the abundance of this species during the last decade. To explain this, data on egg production, which our results show was within the normal range, must be coupled with information on survival of fry and young. Unfortunately, there is no data on survival rates for the period in question and the only conclusion we can make is that survival must have been moderately good to result in the rapid population increase.

The authors wish to thank personnel of the Glenora Fisheries Station, Ontario Department of Lands and Forests, for advice and encouragement and for allowing us to use their accumulated data on white perch. In particular, Mr W. J. Christie, the Director, and Drs D. W. Coble, E. D. Lane, and J. C. McLeod were most helpful.

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REFERENCES

AuClair, Roger P. MS, 1956. The white perch, *Morone americana* (Gmelin), Sebasticook Lake, Maine M.Sc. Thesis. University of Maine, Orono, Maine. 88 p.

Bigelow, H. B., and William C. Schroder. 1953. Fishes of the Gulf of Maine. U.S. Fish Wildlife Serv., Fish. Bull. **53**(74): 405–407.

Conover, N. MS, 1958. Investigations of the white perch *Morone americana* (Gmelin) in Albemarle Sound and the Lower Roanoke River, N.C. M.Sc. Thesis. North Carolina State College, Raleigh, N.C. 58 p.

Henderson, N. E. 1963. Influence of light and temperature on the reproductive cycle of the eastern brook trout, *Salvelinus fontinalis* (Mitchill). J. Fish. Res. Bd. Canada, **20**(4): 859–897.

Hildebrand, S. F., and W. C. Schroeder. 1928. Fishes of Chesapeake Bay. Bull. U.S. Bur. Fish., **43**: 244–247.

Hoover, E. E. 1937. Biological survey of the Androscoggin, Saco and Coastal Watersheds. N.H. Fish Game Dept. Survey Rept. No. 2. 160 p.

MANSUETI, R. J. 1961. Movements, reproduction and mortality of the white perch, *Roccus americanus*, in the Patuxent Estuary, Maryland. Chesapeake Sci., **2**(3–4): 142–204.

1964. Eggs, larvae and young of the white perch, *Roccus americanus*, with comments on its ecology in the estuary. Ibid., **5**(1–2): 3–45.

MILLER, L. W. MS, 1963. Growth, reproduction and food habits of the white perch, *Roccus americanus* (Gmelin), in the Delaware River Estuary. M.Sc. Thesis. University of Delaware, Newark, Del. 62 p.

SCOTT, W. B., AND W. J. CHRISTIE. 1963. The invasion of the lower Great Lakes by the white perch, *Roccus americanus* (Gmelin). J. Fish. Res. Bd. Canada, **20**(5): 1189–1195.

TAUB, S. H. MS, 1966. Some aspects of the life history of the white perch, *Roccus americanus* (Gmelin), in Quabbin Reservoir, Massachusetts. M.Sc. Thesis. University of Massachusetts, Amherst, Mass. 63 p.

THOITS, C. F., III. 1958. A compendium of the life history and ecology of the white perch. Mass. Div. Fish Game, Fish. Bull. No. 24. 16 p.

TRUITT, R. V. 1952. Annual report of the Maryland Department of Research and Education. Educational Series No. 31. Ninth Ann. Rept., Maryland Board Nat. Resources, 1952. 34 p,

Tattoo-Marking of Fingerling Salmonids with Fluorescent Pigments

Tattooing is a useful method for marking fish because a number of different marks may be made by combinations of color, number, and location (Dunstan and Bostick, 1956). Nonfluorescent-pigment tattoos have been retained up to 5 months (Chapman, 1957). Although 5 months is sufficient for many experiments, a longer-lasting pigment would extend the usefulness of the method.

Fluorescent pigments for tattoo-marking of fingerling salmonids were first used in studies of fish mortality in turbines at Shasta Dam.[1] These pigments were first considered by the U.S. Bureau of Commercial Fisheries because of the need for more marks consisting of single tattoos in a standard location on the fish. Marks that require multiple tattoos and multiple locations are more time consuming and susceptible to error. Because fluorescent and nonfluorescent pigments of the same color can be readily differentiated under ultraviolet (UV) light, the number of usable pigments could be doubled by using both types of pigments.

Since 1963, over 100,000 coho salmon (*Oncorhynchus kisutch*), chinook salmon (*O. tshawytscha*), and rainbow trout (*Salmo gairdneri*) have been tattooed with fluorescent pigments by the Bureau for studies on mortality of fingerlings in turbines of hydroelectric dams on the Columbia and Snake rivers. The fluorescent pigments have proved to be reliable, easily recognized marks. These studies, each lasting only a few days, gave no opportunity for determining long-term retention of the tattoos. Therefore, two groups of coho fingerlings were marked with fluorescent-pigment tattoos and held in hatchery ponds to determine the effective life of the marks. Because growth during these tests was small (about 45 mm), the effect of growth on mark visibility was not tested. This note presents the data on long-term retention of tattoo marks.

Materials and methods.— Fluorescent pigments used in this investigation are sold under the trade name of Day-Glo[2] (Switzer Brothers, Inc., 1962). The chemical nature of Day-Glo pigments is described by the manufacturer as "a solid solution of fluorescent dyes in melamine sulfonamide-formaldehyde resin." The resin is a glasslike inert material that completely envelops each particle of fluorescent pigment. The effective life of Day-Glo pigments is

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[1]One of the authors (Donaldson) used fluorescent pigments to mark fingerling salmonids at the Coleman Hatchery, Red Bluff, California, in 1962 for fingerling mortality studies in the turbines of Shasta Dam. The research was sponsored by the U.S. Army Corps of Engineers, Walla Walla District, Walla Walla, Washington.

[2]Trade names referred to in this publication do not imply endorsement of commercial products by the U.S. Bureau of Commercial Fisheries.

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governed by the amount of UV radiation received; the pigments are rated for either 6 or 12 months of outdoor use before noticeable fading. Because water inhibits the penetration of UV rays, tattoo marks of this material on fish are subjected to less exposure and therefore should last longer than the rated life.

The Day-Glo pigments were obtained in insoluble powder form and mixed with water to form a thick paste. Thorough mixing, which is necessary to prevent clogging of the tattoo needles, was difficult by hand stirring, but easy with a blender. The two types of tattooing machines used were described by Chapman (1957) and Volz and Wheeler (1966). The UV light source was a General Electric 15-w "black-light" tube, number F15T8-BLB (3200–4000 Å). When the UV light was on, the fish were examined in an enclosure to shield direct rays of visible light and to increase the brilliance of the fluorescent-pigment tattoos.

Ulceration, a potential cause of fish mortality and mark degeneration, was minimized by adjusting the needles of the marking machines to restrict penetration to skin depth. The first few fish marked were killed and the tattooed portions of the skin carefully separated from the flesh and folded back. Exposure to UV light revealed minute quantities of pigment in the flesh that were invisible under normal light. Needle penetration was considered satisfactory when the tattoo mark was highly visible on the inner surface of the skin, and only minute quantities of pigment (or none) were visible in the flesh.

Two groups of coho fingerlings of the same stock and year-class were marked and held in a pond at the hatchery of the Fish Commission of Oregon at Bonneville Dam, Bonneville, Ore. The first group (group I), marked in December 1963 as underyearlings, consisted of about 500 fish. Group II, marked in April 1964 as yearlings, consisted of about 3000 fish. The Day-Glo pigments injected were aurora pink, signal green, and arc yellow — all rated for 6 months of effective Non-Fade life under outdoor conditions.

In December of 1964, high water conditions in Tanner Creek, which provides water for the hatchery, flushed a considerable portion of the original test population from the holding pond. Periodic checks of mortality did not reveal more than normal hatchery mortality until the fish approached the end of their life expectancy, when mortality increased.

The remaining fish from both groups were examined carefully in July 1965, after group I had been marked 19 months, and group II 15 months. Fish of both groups then averaged 165 mm (fork length) — a growth of about 45 mm from the time of marking for group II, and slightly more than 45 mm for group I.

All fish were first examined under visible light (daylight), and then under UV light. Visibility of the tattoo marks was classed as follows: good — readily visible without close examination; fair — recognizable with close examination; poor — recognizable only with difficulty; and invisible — not recognizable.

Retention of tattoos.— Although many of the tattoos were not visible when viewed in daylight (45.6% from group I and 77.9% from group II), all fish had tattoos that were visible under UV light (Table I). Under UV light, most of the tattoos were classed good (77.2% of group I and 87.0% of group II); the remainder were classed fair or poor.

TABLE I. Percentages of fluorescent-pigment tattoos in various classes of visibility of coho salmon 19 months (group I) and 15 months (group II) after marking as fingerlings.

			Visibility of tattoo marks			
Light source	Color of mark	Total fish	Good	Fair	Poor	Invisible
		19-month group				
Daylight	Green	17	0.0	17.6	23.5	58.8
	Pink	31	9.7	45.2	19.4	25.8
	Yellow	9	11.1	0.0	0.0	88.9
Total/avg		57	7.0	29.9	17.5	45.6
Ultraviolet	Green	17	70.6	23.5	5.9	0.0
	Pink	31	93.5	3.2	3.2	0.0
	Yellow	9	33.3	11.1	55.6	0.0
Total/avg		57	77.2	10.5	12.3	0.0
		15-month group				
Daylight	Green	331	0.0	7.9	4.9	87.3
	Pink	271	1.5	24.7	7.4	66.4
Total/avg		602	0.7	15.4	6.0	77.9
Ultraviolet	Green	331	90.3	7.6	2.1	0.0
	Pink	271	83.0	11.8	5.2	0.0
Total/avg		602	87.0	9.5	3.5	0.0

After the complete check in July 1965, samples of fish from both groups were inspected intermittently. The last sample was taken in May 1966. (An operational breakdown shortly thereafter caused the loss of all fish.) By this time, fish of group I had been marked 29 months, and fish of group II 26 months. All of the 200 fish inspected had visible tattoo marks when viewed under UV light.

The tattoo marks were not completely sloughed by the fingerlings in 19 months and can be expected to remain visible much longer when viewed under ultraviolet light. Retention of fluorescent-pigment tattoos appears to be much greater than that of tattoos of nonfluorescent pigments reported in the literature.

Although the retention of marks reported in these tests was good, growth of the fish may reduce the density and consequently the visibility of the pigment (Kelly, 1967).

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REFERENCES

CHAPMAN, D. W. 1957. An improved portable tattooing device. Progressive Fish-Culturist, **19**(4): 182–184.

DUNSTAN, W. A., AND W. E. BOSTICK. 1956. New tattooing devices for marking juvenile salmon. Fish. Res. Papers, Wash. Dept. Fish., **1**(4): 70–79.

KELLY, WILLIAM H. 1967. Marking freshwater and a marine fish by injected dyes. Trans. Am. Fish. Soc., **96**(2): 163–175.

SWITZER BROTHERS, INC. 1962. Day-Glo daylight fluorescent pigments. Tech. Bull. 1115-B, Switzer Brothers, Inc., 4732 St. Clair Ave., Cleveland, Ohio. 44103. 15 p.

VOLZ, CHARLES D., AND CHESTER O. WHEELER. 1966. A portable fish-tattooing device. Progressive Fish-Culturist, **28**(4): 54–56.

A Subcutaneous Dart Tag for Fish

Preliminary tagging of aquarium-held sablefish (*Anoplopoma fimbria*), English sole (*Parophrys vetulus*), and sockeye salmon (*Oncorhynchus nerka*) was carried out at the Nanaimo Biological Station in 1965 using "grow-thru" dart tags (Fig. 1A–C), applied by tagging knives similar to the one represented in Fig. 2. In operation, the tagging knife with tag in position was immersed in an antibiotic paste prepared by mixing water and Cellosize (Union Carbide) to a consistency of a stiff paste and then adding three powdered antibiotics to a slight excess of saturation: Aureomycin (chlortetracycline) Lederle; Chloramphenicol (chloromycetin) Park Davis; and Terramycin (oxytetracycline) Pfizer. The knife was then inserted under the skin midway between the lateral line and the dorsal fin, just anterior to the front margin of the dorsal fin. The anaesthetic M.S. 222 was used to immobilize the fish prior to tagging.

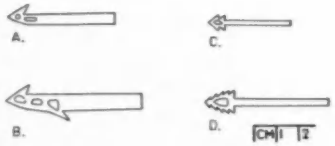

FIG. 1. Types of "grow-thru" tags used to tag: A, 2-year sockeye salmon and sablefish; B, sablefish and English sole; C, sockeye salmon smolts; D, Atlantic salmon.

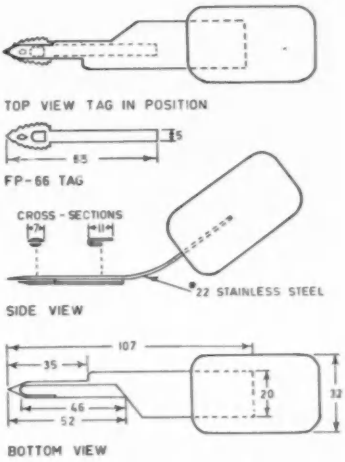

FIG. 2. The FP-66 "grow-thru" dart tag, the tagging knife used to apply it, and an FP-66 tag in position for tagging (dimensions in millimeters).

The tagged fish did not show any signs of behaviour change or infection as a result of the tagging wounds or the effect of carrying the tag.

No "grow-thru" tags were shed by the tagged fish. Sablefish and English sole were handled monthly in dipnets during weighing and measuring. The sockeye salmon were not handled during the experiment. Tags (Fig. 1) were

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retained by all fish, until they were killed, in the following cases: 4 English sole, tag type B, and 6 2-year sockeye salmon smolts, tag type C, for 2 months; 8 2-year sockeye salmon, tag type A, for 3 months; 24 sablefish, tag type B, for 6 months.

Tags dissected from English sole and sockeye smolts after 2 months showed that connective tissue and new flesh had grown through the hole in the tag head and around the barbs of the tag to hold it securely under the skin (Fig. 3). The tags could not be pulled from the specimens without breaking the tag.

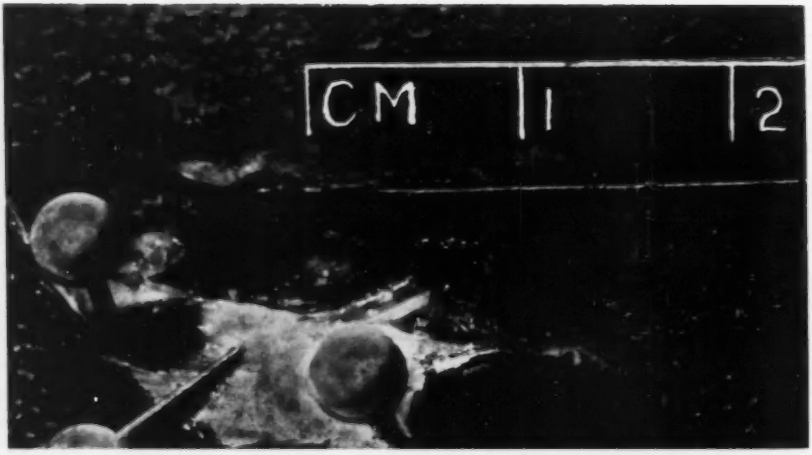

FIG. 3. A "grow-thru" dart tag dissected from an English sole. The pins hold back the skin to show the flesh growing through the hole in the tag head (after 3 months of attachment).

Tags were constructed from vinyl plastic (0.3 mm) with scissors. Numbers were applied with plastic inks to the tag surface.

The 1965 design of the "grow-thru" tag was modified in 1966 by the author and tests were conducted by Symons (MS, 1967) on juvenile Atlantic salmon (*Salmo salar*) at St. Andrews Biological Station. These modified "grow-thru" tags (Fig. 1D) had a much smaller head than the original design; both vinyl (0.3 mm) and mylar (0.08 mm) tags 2–7 cm long and 0.3 cm wide were tested. Symon's experiments showed a retention of 15% on 26 fish and 53% on a second 17 fish after 150 and 103 days, respectively. He concluded from the force needed to remove the tags that growth has occurred through the hole in the head of the tag and suspects that growth occurred before 100 days. The greater loss of tags during the first 15 days and the greater success of the second experiments may suggest improper handling techniques or extensive infection of the tag wound.

Tags similar to those used by Symons were applied to 20 aquarium-held coho salmon smolts (*Oncorhynchus kisutch*) at Robertson Creek by F. Fraser (unpublished data, Institute of Fisheries, University of British Columbia) during November 1966. No tags were shed during the 10 days of the experiment. An antibiotic (Spectrocin) was applied with each tag. The behaviour of the fish was not affected by the tags. Despite the short term of the experiment, dissection by the author revealed that connective tissue had begun to grow through the holes in the tags.

Kennedy (1968) tagged 32 captive sablefish at the Nanaimo Biological Station with the FP-66 "grow-thru" tag (Fig. 3) manufactured commercially (Floy Tag & Manufacturing Inc., Seattle, Wash.). He showed that 16 fish had retained their tags, 6 had lost their tags, and 10 had died of causes unrelated to tagging. In addition, two other tags were lost but were reapplied to the same fish. Kennedy attributes tag loss to handling the fish in nets while weighing them before the tag had become firmly imbedded. He stated that none of the 32 tagged sablefish showed any distress or macroscopic infection, and the tagged fish grew at the same rate as did untagged fish under similar conditions. He reported that of two fish that died 41 days after tagging, tissue had reunited through the large hole in one of the tags, and tissue growth had been initiated in the other. Eight fish that died 56 days after tagging showed tissue reunited through the tag holes in every case.

If antibiotics had been used by Kennedy, growth might have been initiated around the tag sooner to produce better retention. The larger head of the tag and the application of antibiotics may have been a factor in the better retention rates of the "grow-thru" tags used in the initial 1965 experiments. Infection appears to be a problem when tagging freshwater fish with the "grow-thru" tag. This may be alleviated or eliminated with sterile methods and the application of antibiotics to remove infection and thus facilitate rapid growth of the connective tissue around the tag wound. However, Bayliff and Klima (1962) stated that on the basis of their experiments "while antibiotics prevent infection, they actually delay the healing of wounds."

The advantages of a subcutaneous dart tag suggest that further work with antibiotics and growth stimulation chemicals in the tagging would improve, if not overcome, the disadvantage of variable retention in the aquarium experiments. Tags of the "grow-thru" design can be constructed as small as 1 cm long and lettered using 0.08 mylar plastic thus reducing drag and not affecting the behavior of small fish. Preliminary observations suggest good retention in very small fish.

Appreciation is extended to the staff of the Fisheries Research Board of Canada, Biological Station, Nanaimo, B.C., especially to Dr W. A. Kennedy for his encouragement and help in preparing the manuscript, and to Dr G. Bell for assistance in selecting antibiotic

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REFERENCES

BAYLIFF, W. H., AND E. F. KLIMA. 1962. Live-box experiments with anchovetas, *Cetengraulis mysticetus*, in the Gulf of Panama. Intern. Am. Tropical Tuna Comm. Bull., **6**(8): 335–404.

KENNEDY, W. A. 1968. Effect of the FP-66 tag on sablefish growth. J. Fish. Res. Bd. Canada, **25**(10): 2247–2249.

SYMONS, P. E. K. MS, 1967. Retention of Pletcher grow-thru tags applied to young salmon. Fish. Res. Bd. Canada, Tech. Rept. No. 9.

Parasitism of the Toxin-Producing Dinoflagellate *Gonyaulax catenella* by the Endoparasitic Dinoflagellate *Amoebophrya ceratii*

It is due largely to the early work of Chatton (1920) that the dinoflagellate character of many common parasites of planktonic organisms such as the copepods and appendicularians, as well as of unicellular hosts, has been realised. The dinoflagellate character of *Amoebophrya ceratii* (Koeppen) Cachon[1] was only clearly established by the detailed cytological study of this and related species throughout the life-cycle by Cachon (1964). Its convoluted systematic history has been outlined by Chatton and Biecheler (1935). Its known hosts are thecate and nonthecate dinoflagellates. In most cases it has not been possible to determine the species of the host due to morphological aberrations produced by the parasite.

The purposes of this note are to record the presence of this organism outside of European waters in a new, clearly identifiable host, to illustrate its appearance in preserved material so that further records may be forthcoming from routine analyses, and to discuss some features of the host–parasite relationship clearly demonstrated by the material at hand. Furthermore, the involvement of the host species in paralytic shellfish poisoning (its role is summarised by Schantz (1966)) adds to the need for reporting the case, particularly since it suggests a somewhat remote possibility of biological control.

Observations.— The parasite was observed in formalin-preserved samples from three plankton tows made by the College of Fisheries, University of Washington, from Sequim Bay. The latter is a small, shallow bay on the southern side of Juan de Fuca Strait close to its entrance to the Strait of Georgia and Puget Sound. The material was collected in July, September, and October of 1965.

Table I records the dinoflagellate species present. Diatoms were the dominant group in all three samples. In many of the chains of cells of *Gonyaulax catenella* Whedon et Kofoid, morphological aberrations were apparent. Characteristically *G. catenella* shows little size variation within the chains. However, this feature was strikingly altered (Fig. 1, 2) in the Sequim Bay material. The largest cells in the chains reached a diameter of 70–80 μ. A closer examination of the abnormally large cells revealed that each contained a trophont (the feeding stage) of *Amoebophrya ceratii*.

The trophont may be most readily recognised in preserved, unstained material by the presence of a distinctive cavity, the mastigocoele (Fig. 4,

[1]The combination is here attributed to Cachon (1964) who first used it as a deliberate binominal. The combination appears as such in the index to Grassé (1952), but not in the text to which it refers.

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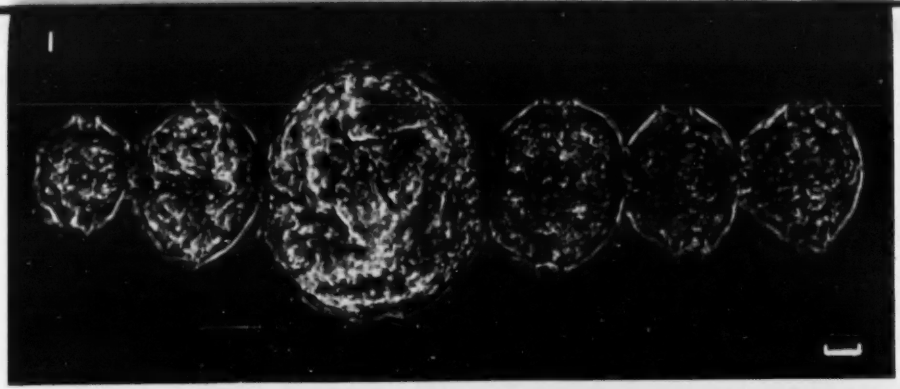
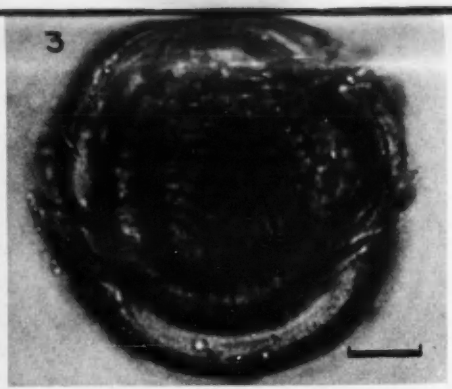
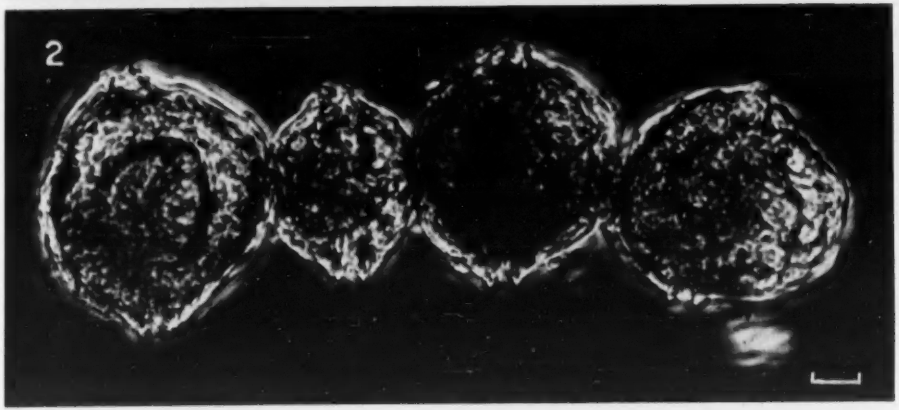
TABLE I. Dinoflagellate composition of Sequim Bay samples, and numbers of taxa of other protist groups.

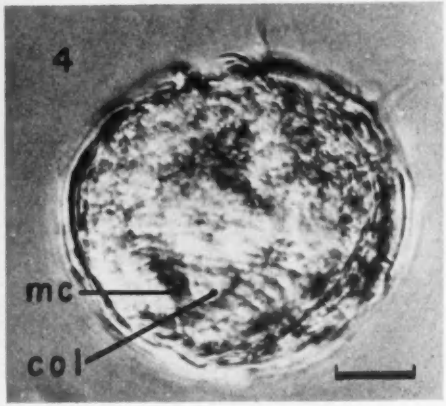
Sample date:	July 27	Sept. 30	Oct. 6
Dinoflagellates:	*Dinophysis acuta* *Entomosigma peridinioides* *Glenodinium pilula* *Gonyaulax catenella* *Gyrodinium contortum* *Oxyphysis oxytoxoides* *Peridinium denticulatum* *P. triquetrum* *P. trochoideum* *Prorocentrum gracile* *Protoceratium reticulatum* (*Amoebophrya caretii*)	*Ceratium fusus* *Dinophysis acuta* *Gonyaulax acatenella* *G. catenella* *G. polygramma* *Gyrodinium fusiforme* *Notiluca scintillans* *Peridinium minisculum* *P. steinii* *P. triquetrum* (*Amoebophrya ceratii*)	*Ceratium fusus* *Dinophysis acuta* *D. lachmannii* *Gonyaulax catenella* *Gymnodinium splendens* *Gyrodinium lachryma* *Notiluca scintillans* *Peridinium crassipes* *P. curvipes* *P. minusculum* *P. pellucidum* *P. steinii* *P. triquetrum* (*Amoebophrya ceratii*)
Diatoms: no of taxa:	10	15	51
Other photosynthetic protists:	2	2	5 (incl. 2 silicoflagellates)
Nonphotosynthetic protists:	3 (incl. 1 tintinnid)	1 (planktonic amoeba)	6 (incl. planktonic amoeba and 3 tintinnids)
Total no. of taxa:	27	29	76

mc), which resembles the classic beehive in shape, its inner and outer surfaces raised in outward-projecting, spiral ridges (Fig. 3). In the chains in which mastigocoeles were apparent they were all orientated in the same direction within the host cells (Fig. 5). As the trophont develops the mastigocoele is gradually compressed until it is virtually invisible and highly sinuous in contour in the mature trophont prior to escape from the host (Cachon, 1964) (Fig. 6). However, the mature trophont is readily recognisable by its considerable expansion of the host cell (Fig. 1) and also by staining. The use of aceto-carmine, which may be employed on formalin-preserved material for crude nuclear observations, revealed that at the mature trophont stage, virtually the entire host cell has a positive reaction (Fig. 6, 7). This is due to the presence within the cell of countless small daughter nuclei of the parasite, budded off in a continuous chain as the parasite develops, their positions corresponding to the ridges on the mastigocoele (Chatton and Biecheler, 1935; Cachon, 1964). The chain begins in the region homologous to the epicone of the parasite, the columella (Fig. 4, *col*). The largest host cells retained their









Scale line on all figures represents 10 μ.

FIG. 1. Six-celled chain of *G. catenella* containing "giant cell" resulting from development of a mature trophont of *A. ceratii*. Phase contrast. July sample.

FIG. 2. Gross morphological aberration of *G. catenella* infested by *A. ceratii*. Cavity formed by parasite distinct in lowermost cell. Phase contrast. July sample.

FIG. 3. Isolated *G. catenella* cell. Focus onto uppermost ridges of the mastigocoele (cavity) of the parasite. Bright field. September sample.

FIG. 4. Median optical section through an early trophont stage of the parasite in an isolated *G. catenella* cell, seen in apical view of the host. Nomarski interference. July sample. *col* = columella, *mc* = mastigocoele.

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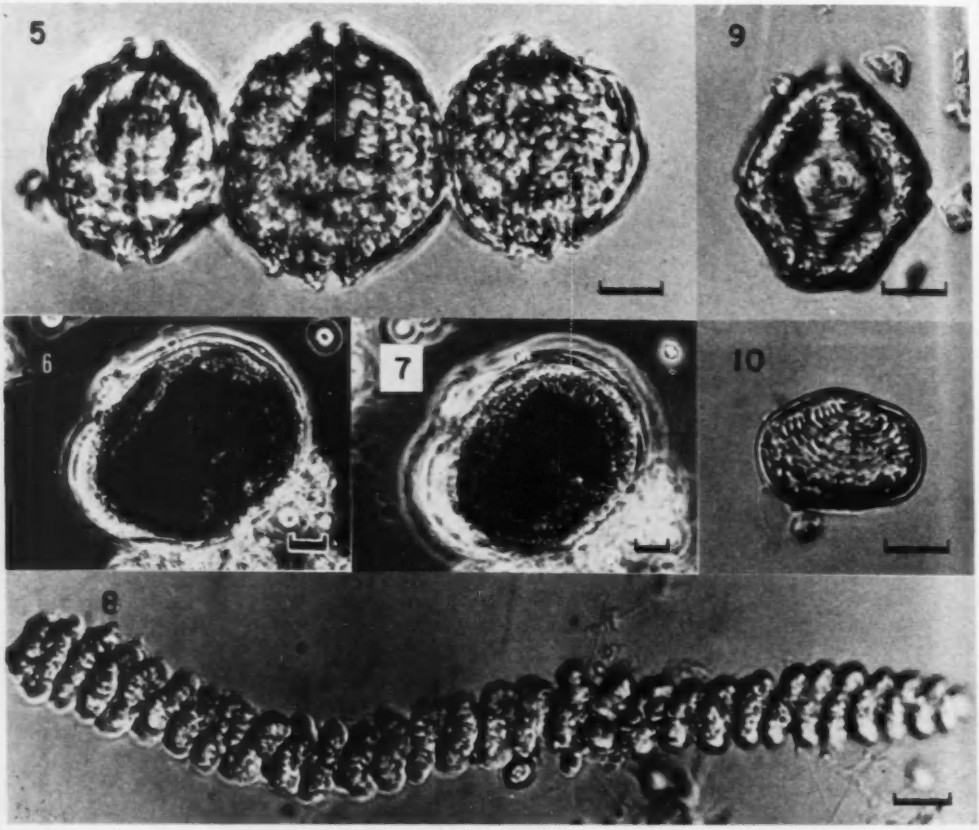

Scale line on all figures represents 10 μ.

FIG. 5. Three-celled chain of *G. catenella* containing developing trophonts in all cells. Note the similarity in orientation of the mastigocoeles in the host cells. Nomarski interference. October sample.

FIG. 6, 7. Two focal depth views of an isolated cell of *G. catenella* containing a mature trophont stage of the parasite. Aceto-carmine stain. Phase contrast. September sample.

FIG. 8. An exceptionally large, intact "vermiforme," the stage of the parasite after exit from the host cell, prior to further fission and liberation of the motile stage. Nomarski interference. October sample.

FIG. 9. Cell of *Peridinium trochoideum* containing a trophont of *A. ceratii*. Nomarski interference. July sample.

FIG. 10. Cyst of indeterminable species of dinoflagellate containing trophont of *A. ceratii*. Nomarski interference. July sample.

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thecae, although Cachon reports that the theca is usually lost as the parasite develops within the host.

Approximately 30–40% of the individuals of *G. catenella* seen were obviously parasitized by *A. ceratii*. The incidence of infestation was probably considerably higher as the initial stages are not recognisable without specific preservation and cytological techniques (Feulgen staining is recommended by Cachon).

One specimen of the stage after escape from the host cell, termed the "vermiforme" by Cachon (1964), was found in the October sample (Fig. 8). This stage has been hitherto found only in cultures. The specimen was exceptionally large (note scale of Fig. 5, 8, 9, and 10). Cachon has pointed out that the size of the parasite is largely determined by its host, suggesting that the vermiform observed did not arise from a parasitized *G. catenella* cell. The "segments" of the vermiform undergo further fission before the free, recognisably dinoflagellate stage, the flagellispore (not observed), is reached.

Of the several hundred dinoflagellate cells infested by *A. ceratii* that were observed (precise numbers not recorded) only two were of different species than *G. catenella*, despite the presence of many other potential hosts (Table I). They were a cell of *Peridinium trochoideum* (Stein) Lemmermann (Fig. 9) and an unidentifiable cystlike cell (Fig. 10). The infestation apparently persisted over a period of 4 months, showing no clear trend in the stages of the parasite observed in the various samples.

Discussion.— The observations have several implications, not only to the understanding of the development and ecology of the parasite, but also, remotely, to public health.

In the development of the parasite, a prominent feature was the constancy of alignment of the mastigocoeles visible in the chain (Fig. 2 and, more clearly, Fig. 5). The cells of *G. catenella* composing a chain are customarily all orientated in the same direction, with the ventral regions of the cells in line with each other (very slight torsion of less than 5° per cell may exist). In the undividing cell the U-shaped nucleus lies in an equatorial plane behind the girdle (Whedon and Kofoid, 1936). In thecate hosts the trophont of *A. ceratii* initially develops within the nucleus of the host (Cachon, 1964). Taken together these features suggest that the alignment of the trophont is very closely determined by the alignment of the host nuclear material.

The presence of unruptured thecae in even the most grossly swollen host cells showed that the thecae grew in pace with the developing trophonts. The basic plate pattern was unchanged, suggesting that genetic control of the thecal pattern still existed despite digestion of the host nucleus by the parasite. It should be recalled, however, that *G. catenella* appears to be exceptional among the hosts of *A. ceratii* in this respect.

To date virtually all records of species of *Amoebophrya* have been limited to the Mediterranean and other European waters. This appears now to be largely a result of a lack of awareness of (or interest in) the group by all but a few French protistologists. Balech (1963, fig. 37) has unwittingly illustrated

a mastigocoele within *Blepharocysta splendor-maris* Ehrenberg, but the locality is not given. In addition to the Sequim Bay material, the present author has observed *A. ceratii* within a few unidentifiable cysts in the Andaman Sea. Consequently its distribution appears to be fairly cosmopolitan.

The strong host preference shown by *A. ceratii* for *G. catenella* in the Sequim Bay community, although not absolute, has a particularly interesting implication. The latter is apparently responsible for the majority of cases of paralytic shellfish poisoning on the west coast of North America, the only other source of toxin in this region being the closely related species *G. acatenella* Whedon et Kofoid, implicated in one case to date[2] (Prakash and Taylor, 1966). As yet no means have been found to prevent populations of this species, or for that matter, any other dinoflagellate, from developing into blooms in natural waters. The most extensive attempt at control of a dinoflagellate has been that of Marvin and Proctor (1966) who tested 4306 compounds in an attempt to discover a selective chemical control agent for *Gymnodinium breve* Davis. Only five compounds had the necessary toxicity for *G. breve*, and they were prohibitively expensive for use in the field where *G. breve* causes fish mortalities.

It seems theoretically possible, therefore, that the answer may ultimately lie in the use of a biological control agent, similar to *A. ceratii*. *Amoebophrya ceratii* itself was not totally fatal to its host population in the present case. However, there is no means of knowing to what extent it modified the growth of the population. The difficulties of rearing large quantities of the parasite for seeding would be great, but *A. ceratii* has been maintained in culture by Chatton and Biecheler (1935) and by Cachon (1964).

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REFERENCES

BALECH, E. 1963. La familia Podolampacea (Dinoflagellata). Bol. Inst. Biol. Maritima, Univ. Nacion. Buenas Aires, **2**: 33 p.

CACHON, J. 1964. Contribution à l'étude des Péridiniens parasites. Cytologie, cycles évolutifs. Ann. Sci. Nat. Zool., 12e Ser., **6**: 1–158 + 36 pl.

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[2]*Gonyaulax polyedra* Stein, a cause of mass marine fauna mortalities off California, has been shown to produce a mild paralytic shellfish toxin in the laboratory (Schradie and Bliss, 1962).

CHATTON, É. 1920. Les Péridiniens parasites. Morphologie, reproduction, éthologie. Arch. Zool. Exptl. Gen., **59**: 1–475.

CHATTON, É, AND B. BIECHELER. 1935. Les *Amoebophrya* et le *Hyalosaccus*; leur cycle évolutif. L'ordre nouveau des Coelomastigina dans les Flagellés. Compt. Rend., **200**: 505–507.

GRASSÉ, P.-P. 1952. Traité de Zoologie. Anatomie, systématique, biologie. Masson et Cie, Paris. 1071 p.

MARVIN, K. T., AND R. R. PROCTOR, JR. 1966. Laboratory of redtide control agents. U.S. Fish. Wildlife Serv. Fish. Bull., **66**(1): 163–164.

PRAKASH, A., AND F. J. R. TAYLOR. 1966. A "red water" bloom of *Gonyaulax acatenella* in the Strait of Georgia and its relation to paralytic shellfish toxicity. J. Fish. Res. Bd. Canada, **23**(8): 1265–1270.

SCHANTZ, E. J. 1966. Biochemical studies on certain algal toxins. p. 51–65. *In* R. I. Mateles and G. N. Wogan [ed.] Biochemistry of some microbial toxins. M.I.T. Press, Cambridge, Mass, and London. 171 p.

SCHRADIE, J., AND C. A. BLISS. 1962. The cultivation and toxicity of *Gonyaulax polyedra*. Lloydia, **25**(4): 214–221.

WHEDON, W. F., AND C. A. KOFOID. 1936. Dinoflagellata of the San Francisco region. 1. On the skeletal morphology of two new species, *Gonyaulax catenella* and *G. acatenella*. Univ. Calif. Publ. Zool., **41**(4): 25–34.

Effect of the FP-66 Tag on Sablefish Growth

The effect of FP-66 tags on the growth of sablefish *Anoplopoma fimbria* was assessed in connection with the development of techniques for growing them in captivity at the Nanaimo Biological Station. The FP-66 tag was developed by Mr F. T. Pletcher (Pletcher, 1968). It is like the tag illustrated in Pletcher's Fig. 2.

The sablefish used had all been captured by trawls and had been held for just over a month prior to tagging, except for two that had been held for 10 days. For tagging they were heavily anaesthetized with M.S. 222 then laid on wet paper towels placed on a flat surface. The tag was inserted on the left side below the origin of the first dorsal fin, halfway between fin and lateral line. Tags were disinfected and the tagging knife was dipped in disinfectant between operations. The wound was not treated. I considered it important that the tag be inserted just under the skin rather than deep into the musculature. None of the sablefish used in this experiment, or tagged by this technique for other purposes (32 in all) showed any distress or macroscopic evidence of infection of the tagging cut. Two sablefish were examined 41 days after tagging; in one the tissue had reunited through the larger hole in the tag, in the other it had not quite reunited. Eight sablefish were examined 56 days after tagging; tissue had reunited through both holes of the tag in every case, including one case where the tag had almost pulled out of the tagging cut and where scar tissue at the surface of the cut had grown through the larger hole in the tag.

The experiment was conducted in six identical oval fibreglas tanks each of about 625 liters capacity and supplied continuously with sea water pumped from Departure Bay at varying salinities and temperatures. On April 25, 1967, 13 sablefish were tagged with FP-66 tags and distributed approximately evenly among four of the tanks to minimize crowding during a recovery period, since contact between fish might loosen newly applied tags. At the same time 10 sablefish of comparable size were put aside as future controls in the other two tanks.

The experiment is regarded as starting on May 30 when the fish were rearranged to give: four groups, each with two tagged, two untagged; a group with one untagged, three tagged; and a group with one untagged, two tagged. The unbalanced numbers resulted from failure to procure enough sablefish to set up the experiment as originally planned.

As a result of water supply failure, all the fish in one of the tanks — two tagged and two untagged — died on June 5. During the experiment three tagged fish, one in each of three tanks, lost their tags but each was still distinguishable by a scar at the tagging site.[1] There were eight untagged controls

[1]The tags were apparently lost because the fish were handled several times after tagging. There is every reason to believe that undisturbed sablefish would retain FP-66 tags quite well.

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and the eight tagged fish which had retained their tags when the experiment was terminated on November 15, 1967.

The fish were weighed at the beginning and end of the experiment and at three intermediate dates on a spring balance which could be read to the nearest 10 g. They were first anaesthetized with M.S. 222 and the weight was taken after they had drained for a fixed period as determined by stop watch.

Mean weights of the untagged and of the tagged fish which retained their tags at each weighing during the experiment, as well as on April 25, are compared in Table I. The difference between mean weights of tagged and untagged fish changed little during the experiment, indicating that tagged and untagged sablefish did not differ appreciably in growth rate.

TABLE I. A comparison between weights in grams of eight tagged and eight untagged sablefish at successive weighings.

	Apr. 25	May 30	July 18	Aug. 15	Oct. 17	Nov. 15
Tagged fish						
Mean wt	768	815	1106	1169	1536	1640
Standard deviation	822	861	979	990	1048	1073
Increment during experiment	–	0	291	354	721	825
Untagged fish						
Mean wt	675	719	1000	1066	1438	1538
Standard deviation	525	551	536	659	638	667
Increment during experiment	–	0	281	347	719	819
Difference between mean wt of tagged and untagged	93	96	106	103	98	102

Had the combined weights of all 11 fish tagged originally been considered instead (in which case original mean weights of tagged and untagged fish would have been more nearly the same), exactly the same conclusion would have resulted.

In three tanks the untagged gained more than the tagged; in two they gained less. If, however, the fish which lost their tags are included, the untagged gained more than the tagged in two tanks and they gained less in three tanks. Thus consideration of gains in individual tanks supports the conclusion that there was essentially no difference in growth rate attributable to the tag.

Even during the period allowed for recovery from tagging, i.e. April 25 to May 30, the tagged fish seemed to grow at essentially the same rate as the untagged. However, note that the controls were not in the same tanks as the tagged fish during the period and that there were more controls per tank.

All the evidence supports the conclusion that the FP-66 tag does not affect the growth rate of sablefish in captivity.

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REFERENCES

PLETCHER, F. T. 1968. A subcutaneous dart tag for fish. J. Fish. Res. Bd. Canada, **25**(10): 2237–2240.

Delay of Moult by the Larvae of *Homarus americanus*

The larvae of some marine invertebrates delay settling and metamorphosis if a suitable environment for the adult is unavailable (Barnes, 1967). The adaptive value may be that permanent association with a suitable environment is more beneficial to the species than a shorter pelagic life and its lower risk of predation. This seems a logical conclusion when considering sessile organisms. But for mobile animals such as decapod crustaceans, it may be more beneficial to settle anywhere and then move to a suitable environment.

In this experiment I tried to determine if there is a difference in time of moult when the larvae of *Homarus americanus* are presented with different bottom conditions. The lobster does not have a clearly marked metamorphosis which could be used as an end point for the larval stage. However, the fifth stage, beginning after the fifth moult, is considered to be the termination of the larval period and the beginning of the fully epibenthic life of the juvenile lobster (Hughes and Matthiessen, 1962). The fifth moult is therefore analogous to settling and metamorphosis. At 20 C the first three stages last a total of about 12 days during which the larvae are completely pelagic. The fourth larval stage is a period of both pelagic and epibenthic life and lasts about 12 days at 20 C (Herrick, 1909).

The time from fourth to fifth moult was measured when randomly assigned larvae were subjected to each of four bottom conditions: mud, sand, gravel (2–4 cm diam), and bare. Particle size for mud and sand was not determined. Five larvae were put into each of 24, 14-quart plastic dishpans immersed in a running seawater bath to maintain equal temperature. The experiment was run twice during the period June 28–July 31, 1967, making a total of 240 larvae started. The larvae were obtained a day after moulting into the fourth stage and were put immediately into the test pans in the first run. In the second run they were not subjected to the test conditions until day 8. Up to this time they were kept in a 20-gal aquarium with a bare bottom. The light cycle was 14.5 hr light and 9.5 hr dark. The larvae were fed brine shrimp nauplii once daily. Temperature in the first run averaged 20.5 C (19–22 C) and in the second run 22.6 C (22–26 C). The *p*H of the water was not measured during the first run but at the end of the second run the *p*H was not different among treatments. Due to cannibalism and possibly other types of mortality, only a total of 67 (55.8%) in the first run and 87 (72.5%) in the second run survived to the fifth stage when 30 individuals were started under each condition. An analysis of variance showed no differential survival attributable to substrate differences.

The data were pooled and the results of both runs are shown in Table I and Fig. 1. The larvae on gravel moulted 2.0 days earlier than those on

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TABLE I. Number of lobsters moulting into fifth stage, mean day of moult, and standard deviation for fourth stage lobsters raised on four different bottom conditions.

	N	$\bar{X}$	S
Gravel	36	14.1	1.40
Sand	38	15.8	1.72
Bare	33	15.9	1.84
Mud	36	16.2	1.88

sand and 2.7 days earlier than those on mud and bare conditions if one measures the time at the point which 50% of the surviving larvae have passed the fifth moult (Fig. 1).

The mean day of moult of those larvae which had moulted by day 19 in run one when the run was terminated, and by day 20 when all but one larva had moulted in run two showed a difference of between 1.7 and 2.1 days in a comparison of gravel to other conditions (Table I). Duncan's new multiple range test (Steele and Torrie, 1960) showed the mean day of moult on gravel to be significantly different at the .05 level from the mean day of moult under all other conditions.

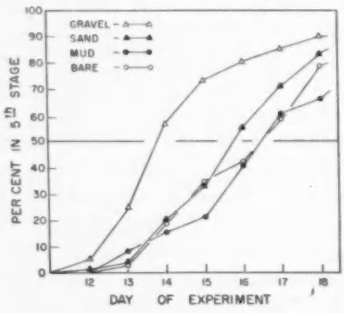

FIG. 1. Percent lobster larvae in fifth stage on subsequent days when subjected to different bottom conditions: gravel, sand, mud, and bare. Differences in time of moult can be measured at the point where 50% of the larvae have achieved the fifth stage.

Herrick (1909) claims the fourth larval stage to be one of irregularly pelagic and epibenthic life; this would be consistent with a hypothesis that the larvae are testing the bottom. My observations of larval movements coincide with Herrick's. Delay of moulting when exposed to unsuitable bottom conditions means that the larvae may remain pelagic for a longer than average period when swimming over less favorable bottoms and descend to the bottom permanently when favorable (rocky) conditions are reached. Tests are underway to examine the hypothesis that one of the factors determining early settling is the availability of crevices in the gravel.

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REFERENCES

BARNES, H. 1967. Ecology and experimental biology. Helgolander Wiss. Meeresuntersuch., **15**: 6–26.

HERRICK, F. H. 1909. Natural history of the American lobster. Bull. U.S. Bur. Fish., **29**: 147–408.

HUGHES, J. T., AND G. C. MATTHIESSEN. 1962. Observations on the biology of the American lobster, *Homarus americanus*. Limnol. Oceanog., **7**: 414–421.

STEELE, R. G. D., AND J. H. TORRIE. 1960. Principles and procedures of statistics. McGraw-Hill, New-York. 481 p.

RECENT ARTICLES ON INVESTIGATIONS OF THE FISHERIES RESEARCH BOARD OF CANADA PUBLISHED IN OTHER PERIODICALS

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1245. INGLES, C. J., J. R. TREVITHICK, M. SMITH, AND G. H. DIXON. 1966. Biosynthesis of protamine during spermatogenesis in salmonid fish. Biochem. Biophys. Res. Commun., **22**(6): 627–634.

1246. BORDEN, R. KEITH, AND MICHAEL SMITH. 1966. Nucleotide synthesis. III. Preparation of nucleoside-3′,5′ cyclic phosphates in strong base. J. Org. Chem., **31**: 3247–3253.

1247. BORDEN, R. KEITH, AND MICHAEL SMITH. 1966. Nucleotide synthesis. II. Nucleotide *p*-nitrophenyl and 2,4-dinitrophenyl esters. J. Org. Chem., **31**: 3241–3246.

1248. BILINSKI, E., N. J. ANTIA, AND Y. C. LAU. 1968. Characterization of phospholipase C from a marine planktonic alga (*Monochrysis lutheri*). Biochim. Biophys. Acta, **159**: 496–502.

1249. YAMAZAKI, FUMIO, AND EDWARD M. DONALDSON. 1968. The spermiation of goldfish (*Carassius auratus*) as a bioassay for salmon (*Oncorhynchus tshawytscha*) gonadotropin. Gen. Comp. Endocrinol., **10**(3): 383–391.

1250. BROCKERHOFF, H. 1968. Substrate specificity of pancreatic lipase. Biochim. Biophys. Acta, **159**: 296–303.

1251. IDLER, D. R., AND D. A. HORNE. 1968. Recovery of microquantities of steroids from silica gel following repeated thin-layer chromatography. Steroids, **11**(6): 909–914.

1252. SPRAGUE, JOHN B. 1968. Avoidance reactions of rainbow trout to zinc sulphate solutions. Water Res., **2**: 367–372.

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